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Factors Predicting Progression of Chronic Kidney Disease in IgA Nephropathy

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Front cover: Ladewig's Trichrome-stained renal biopsy specimen, showing advanced chronic glomerular and tubulo-interstitial lesions in IgA nephropathy, photograph taken by Dr Georg Jaremko, pathologist, Karolinska University Hospital.

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”Mot det förgångna: tack,
till det kommande: ja!”

(“Towards the past: thank you,
to the future: yes!”)

Excerpt from the Haiku poem by Dag Hammarskjöld (1905-1961)

ABSTRACT

Glomerulonephritis (GN) is the main cause of chronic kidney disease (CKD) in patients on renal replacement therapy, with IgA nephropathy (IgAN) being the most frequent single diagnosis. Patients with reduced kidney function and/or proteinuria have an increased risk of premature cardiovascular disease (CVD). Based on the assumption that progression of atherosclerosis and glomerulosclerosis share similar pathogenetic mechanisms, the aim of this thesis was to investigate to which extent some of the described traditional and non-traditional cardiovascular risk factors are also predictors of renal outcome in patients with a homogenous renal diagnosis, e.g. IgAN. Chronic inflammation is supposed to be one of the driving forces in renal disease progression, and disturbances in both innate and adaptive immunity seem to be involved in the pathogenesis of IgAN, with T-lymphocytes being of particular interest. We therefore also investigated the prognostic impact of plasma levels of soluble interleukin-2 receptor, suggested as a marker of continuous T-cell stimulation.

Study I was a pilot study, investigating activated monocytes (MOs) as one possible source of oxidative stress in IgAN. Peripheral blood MOs from 16 patients with IgAN and 16 healthy controls were stimulated *in vitro* and the production of reactive oxygen species (= respiratory burst) was measured by flow cytometry. In the patients, this was repeated after one month's treatment with 20 mg atorvastatin. At baseline, the respiratory burst of *in vitro* stimulated MOs was higher in patients as compared to in healthy controls. After atorvastatin treatment, there was a significant reduction of unstimulated and stimulated MO respiratory burst compared to baseline values.

In **study II**, the apolipoprotein B /apolipoprotein A-I ratio (apoB/apoA-I), indicating the balance of atherogenic and anti-atherogenic lipids, was analyzed in 70 IgAN patients with CKD stage 1-3 (estimated glomerular filtration rate (eGFR) $\geq$ 30ml/min/1.73m²) and in 70 age- and gender matched healthy control subjects. Patients with IgAN had higher serum levels of apoB/apoA-I compared to the controls, and an apoB/apoA-I ratio greater than the proposed threshold value of 0.9 for men and 0.8 for women was associated with an increased risk for the development of end stage renal disease (ESRD) in the patients, after a median follow-up period of 3.8 years.

Study III comprised 194 patients with IgAN, of whom 179 with CKD stage 1-4 (eGFR $\geq$15ml/min/ 1.73m²) had been prospectively followed for up to 16 years (median 52 months). Plasma levels of soluble interleukin-2 receptor alfa unit (sIL-2Ra) were higher in IgAN patients compared to in 84 healthy controls. In survival analysis, baseline sIL-2Ra levels in the upper tertile predicted worse renal outcome in IgAN patients. Soluble IL-2Ra also correlated to the rate of renal function decline (annual eGFR slope). In 51 patients, in whom the renal biopsy had been scored according to the new Oxford classification, higher IL-2Ra levels were associated with the presence of more than 25% tubular atrophy/ interstitial fibrosis (T score 1-2), after adjustment for renal function.

Study IV was performed in 180 IgAN patients with CKD stage 1-4, followed for a median of 55 months. Baseline serum levels of fibroblast growth factor 23 (FGF23), a key player in the chronic kidney disease-mineral and bone disorder (CKD-MBD), were associated with severe renal outcome and with the annual eGFR slope. Moreover, the FGF23 level at baseline correlated to the degree of albuminuria at baseline and to time-averaged albuminuria during follow-up, a new finding that implicates possible direct effects of FGF23 on the glomerular filtration barrier.

In **Summary**, several factors involved in the progression of atherosclerosis are also present and predictive of renal outcome in patients with IgAN, independent of the confirmed main risk factors: proteinuria and hypertension. Furthermore, continuous T-cell stimulation may contribute to renal disease progression in IgAN. The investigated potential biomarkers could be useful in the monitoring of the therapy.

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LIST OF PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals

I. **Lundberg S**, Lundahl J, Gunnarsson I, Jacobson SH. Atorvastatin-induced modulation of monocyte respiratory burst in vivo in patients with IgA nephropathy: a chronic inflammatory kidney disease. Clin Nephrol. 2010; 73:221-8

II. **Lundberg S**, Gunnarsson I, Jacobson SH. Impact of apolipoprotein B/ apolipoprotein A-I ratio on renal outcome in IgA nephropathy. Scand J Urol Nephrol. 2012; 46:1-8

III. **Lundberg S**, Lundahl J, Gunnarsson I, Sundelin B, Jacobson SH. Soluble interleukin-2 receptor alfa predicts renal outcome in IgA nephropathy. Nephrol Dial Transplant. (2011) doi:10.1093/ndt/gfr554

IV. **Lundberg S**, Qureshi AR, Olivecrona S, Gunnarsson I, Jacobson SH, Larsson TE. FGF23, albuminuria and disease progression in patients with chronic IgA nephropathy. Clin J Am Soc Nephrol. (2012) doi:10.2215/CJN/10331011

Other publications based on the patient cohort, not included in this thesis

Vuong MT, **Lundberg S**, Gunnarsson I, Wramner L, Seddighzadeh M, Hahn-Zoric M, Fernström A, Hanson LA, Do LT, Jacobson SH, Padyukov L. Genetic variation in the transforming growth factor-beta 1 gene is associated with susceptibility to IgA nephropathy. Nephrol Dial Transplant. 2009; 24:3061-7.

Vuong MT, Gunnarsson I, **Lundberg S**, Svenungsson E, Wramner L, Fernström A, Syvänen AC, Do LT, Jacobson SH, Padyukov L. Genetic risk factors in lupus nephritis and IgA nephropathy – no support of an overlap. PLoS One. 2010; 5:e10559.

Vuong MT, Hahn-Zoric M, **Lundberg S**, Gunnarsson I, van Kooten C, Wramner L, Seddighzadeh M, Fernström A, Hanson LA, Do LT, Jacobson SH, Padyukov L. Association of soluble CD89 levels with disease progression but not susceptibility in IgA nephropathy. Kidney Int. 2010; 78:1281-7.

CONTENTS

LIST OF ABBREVIATIONS

ACEI	Angiotensin converting enzyme inhibitor
AOPP	Advanced oxidation protein products
APO	Apolipoprotein
ARB	Angiotensin II receptor blocker
aHTs	Antihypertensives
BM	Basement membrane
BMI	Body mass index (kg/m²)
BP	Blood pressure
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C- reactive protein
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
ESRD	End-stage renal disease
eGFR	Estimated glomerular filtration rate (ml/min/1.73m²)
FACS	Fluorescence-activated cell sorting
FGF	Fibroblast growth factor
fMLP	N-formylmethionyl leucyl phenylalanine
FU	Follow-up
Gd-IgA1	Galactose-deficient IgA1
GN	Glomerulonephritis
HDL	High-density lipoprotein
HR	Hazard ratio
ICAM-1	Serum intercellular adhesion molecule-1
IgA	Immunoglobulin A
IgAN	Immunoglobulin A nephropathy
IGF-1	Insulin-like growth factor-1
IL-2Ra	Interleukin-2 receptor alpha
KDOQI	Kidney disease outcomes quality initiative
LDL	Low-density lipoprotein
MAP	Mean arterial pressure
MCP-1	Monocyte chemoattractant protein-1
MDRD	Modification of diet in renal disease (study and equation)
MFI	Mean fluorescence intensity
MIF	Macrophage inhibitory factor
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor κ-light-chain-enhancer of activated B cells
NGAL/MMP-9	Neutrophil gelatinase-associated lipocalin/ matrix metalloproteinase-9 complex
ox-LDL	Oxidized low-density lipoprotein
PDGF	Platelet derived growth factor
PMA	Phorbol-12-myriastate-7-acetate
PTH	Parathyroid hormone
RAAS	Renin angiotensin aldosterone system
RANTES	Regulation on activation, normal T expressed and secreted

ROS	Reactive oxygen species
RR	Risk ratio
RRT	Renal replacement therapy
SD	Standard deviation
TA	Time-averaged
TGFβ	Transforming growth factor beta
TLR	Toll-like receptor
TNF	Tumor necrosis factor
sTNFR	Soluble tumor necrosis factor receptor
U-alb/cr	Urine- albumin/creatinine ratio

BACKGROUND

1.1 INTRODUCTION

A diagnosis of chronic kidney disease (CKD) is made, irrespective of cause, based on one of the following criteria proposed in 2002 by the National Kidney Foundation and Kidney Disease Outcomes Quality Initiative (NFK-KDOQI) and endorsed by the Kidney Disease: Improving Global Outcomes (KDIGO) in 2004 (1, 2):

1. **Kidney damage for ≥ 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifest by *either*:**
    a. **Pathologigal abnormalities; or**
    b. **Markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests**
2. **GFR < 60mL/min/1.73m² for ≥ 3 months, with or without kidney damage**

The prevalence of CKD in the general population is appreciated to be about 10-13% (3-5) with a greater risk in individuals with a family history of CKD (6). Patients suffering from CKD have an increased risk of death due to cardiovascular disease (CVD) such as myocardial infarction, heart failure, sudden cardiac death or stroke, with the highest risk in those who develop end stage renal disease (ESRD) requiring chronic dialysis or a kidney transplant (7, 8).

CKD is currently classified by the five stages defined in 2002, which reflect patient prognosis and should lead to recommended clinical action plans to prevent CKD progression and to reduce the risk of CVD (Table 1) (1).

Table 1. Classification of CKD stages and recommended clinical action plan

Stage	Description	GFR (mL/min/1.73m²)	Action
	At increased risk	≥ 90 (with CKD risk factors)	Screening, CKD risk reduction
1	Kidney damage with normal or ↑ GFR	≥ 90	Diagnosis and treatment, Treatment of comorbid conditions, slowing progression, CVD risk reduction
2	Kidney damage with mild ↓ GFR	60 - 89	Estimating progression
3	Moderate ↓ GFR	30 - 59	Evaluating and treating complications
4	Severe ↓ GFR	15 - 29	Preparation for kidney replacement therapy
5	Kidney failure	< 15 (or dialysis)	Replacement (if uremia present)

The high prevalence of CVD among incident dialysis patients suggests that pathologic cardiovascular changes begin in earlier stages of CKD, and that implementation of risk factor reduction strategies earlier in the course of CKD may provide an opportunity to prevent CVD. The risk factors for CKD and CVD are, in many instances, the same and it is generally assumed that atherosclerosis and glomerulosclerosis display similar pathogenetic mechanisms (9-11). However, the presence of traditional risk factors for CVD, such as old age, smoking, hypertension, diabetes mellitus, dyslipidemia, and left ventricular hypertrophy, as described primarily in the Framingham study, does not seem to provide a satisfactory explanation for the huge cardiovascular burden in the CKD population. Other so-called non-traditional risk factors, such as chronic inflammation, oxidative stress, altered calcium-phosphate metabolism and malnutrition seem to appear early during the process of CKD and to contribute to the significant difference in cardiovascular mortality between individuals with CKD and those without (12-14).

There is emerging evidence for lower estimated glomerular filtration rate (eGFR) and higher albuminuria as being independent risk factors for CVD, mortality, progressive CKD and ESRD (15-17). As a consequence, the recent work of a KDIGO initiated Controversies Conference has led to the proposal of a **modified classification of CKD**, **adding albuminuria stage** (**A1:** optimal and high-normal, albumin-to-creatinine ratio (ACR) < 30 mg/g; **A2:** high, ACR 30 – 299 mg/g; **A3**: very high and nephrotic, ACR > 300 mg/g), **subdividing CKD stage 3 in 3a** (eGFR 45 -59 ml/min/1.73m²) **and 3b** (eGFR 30 – 44 ml/min/1.73m²) and **emphasizing the clinical diagnosis** (diabetes, hypertension, glomerular disease, many others, transplant, unknown) (18). The threshold of eGFR 45 ml/min/1.73m² for subdividing CKD stage 3 was chosen due to the substantial increase in risk of cardiovascular morbidity and mortality found in patients with renal function below this value (19).

The proteinuric diseases glomerulonephritis, diabetic nephropathy and hypertensivenephrosclerosis are all major causes of CKD leading to ESRD. Glomerulonephritis is the main cause of CKD in patients treated with dialysis or a kidney transplant in the Swedish population (Swedish Renal Registry, www.snronline.se). Among those, IgA nephropathy (IgAN) is the most frequent diagnosis of glomerulonephritis, confirmed by renal biopsy.
In patients with IgAN, an increased risk of developing CVD compared to age-matched individuals in the general population has been shown by Myllymaki et al (20).

The aim of the studies in this thesis was to investigate to what extent some of the described traditional and non-traditional cardiovascular risk factors are also present and are predictors of renal outcome in patients with IgAN.

1.2 IGA NEPHROPATHY

IgA nephropathy was described histologically for the first time in 1968 by Berger and Hinglais by its characteristics of *dépôts intercapillaires d'IgA-IgG* (intercapillary deposits of IgA-IgG) (21). The diagnosis also requires the presence of mesangial proliferative changes.

1.2.1 Epidemiology

IgA nephropathy is the most common form of primary glomerulonephritis world-wide with an annual incidence rate of at least 25/million in adults, varying between different countries (22, 23). There are no discernible trends in incidence rates over time. The disease can exist subclinically and is therefore only detected by chance in some patients (24). Racial differences and referral policies for diagnostic biopsy can affect the incidence rates found. In a screening study among children (0-15 years) in Japan 45 children/million/year were diagnosed with IgAN (25). Moreover, 16% of 510 kidneys from apparently healthy kidney donors showed histopathological changes diagnostic for IgAN (26). There is a peak incidence in the second and third decades of live and men are more frequently affected by IgAN than women, with a ratio ranging from 2:1 to 6:1 (27).

1.2.2 Clinical presentation

The archetypical clinical presentation of IgAN is that of a young male patient with episodic macroscopic hematuria during an upper respiratory tract infection, which is the presenting feature in 30 – 40% of cases. Such episodes usually recur for some years but disappear over time. Another 30 – 40% of patients with IgAN are identified by asymptomatic urine abnormalities. Only a few patients have proteinuria without microscopic hematuria. About 5% of all patients develop a nephrotic syndrome that can appear both together with mild glomerular injury and in advanced glomerulosclerosis. Less than 5% of all patients with IgAN present with acute renal failure due to crescentic nephritis or due to tubular occlusion in heavy glomerular hematuria (28). The remaining patients with IgAN already have renal impairment and hypertension, sometimes of a malignant nature, at the time of diagnosis, presumably due to longstanding unrevealed disease.

1.2.3 Pathogenesis

The broad spectrum of clinical pictures and the varying associations found with genetic risk alleles among different populations make IgAN appear to be a clinicopathological phenotype, and the result of a variety of etiologic and pathogenetic factors (24). Interestingly, IgA nephropathy in the ddY mouse model shows similar variability, including asymptomatic, early and late-onset overt disease (29). Most probably, there is a hereditable component in IgAN, but additional factors are needed to produce overt renal disease.

It has been shown that four main processes contribute to the development of IgAN:

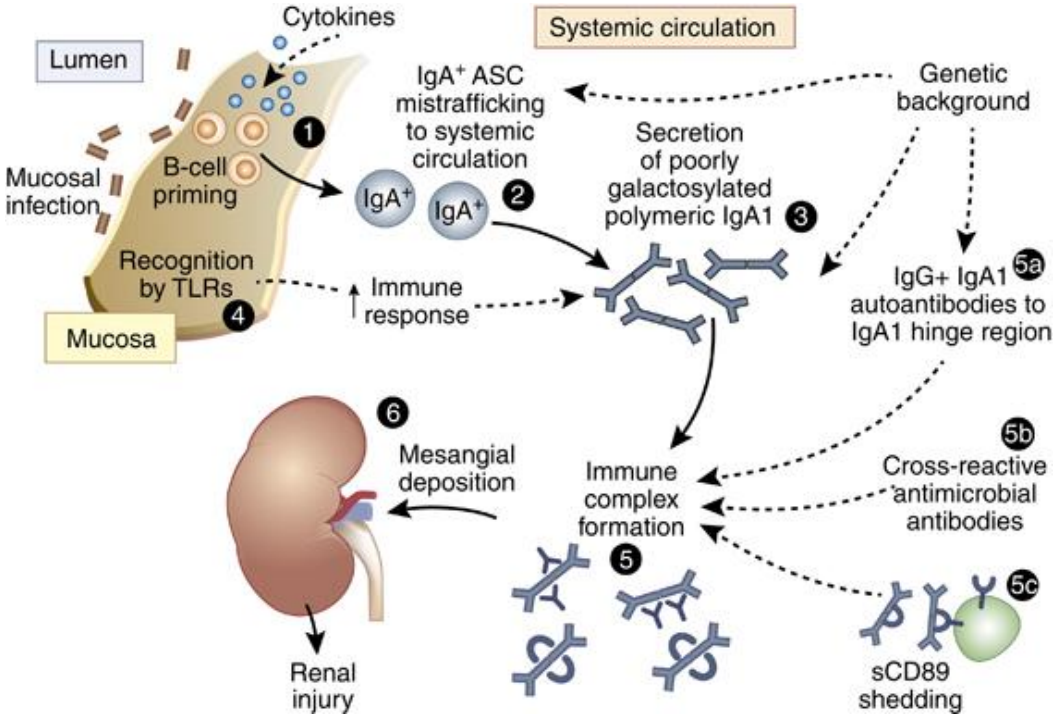
1. A genetically determined increase in circulating levels of galactose-deficient IgA1 (Gd-IgA1)

2. Synthesis of IgG and IgA antibodies directed against Gd-IgA1

3. Formation of circulating, polymeric Gd-IgA1 containing immune-complexes

4. Deposition of these immune-complexes in the kidney, leading to activation of mesangial cells, characterized by proliferation and secretion of extracellular matrix, cytokines and chemokines which further promote the renal injury (30-33).

How these processes may interact with other pathogenetic mechanisms, as described below, is illustrated in a recently published review article by Boyd et al (Figure 1) (34).



<u>Figure 1. An overview of the pathogenesis of immunoglobulin (Ig)A nephropathy.</u> (1) Mucosal infection primes naive B cells to class switch to become IgA$^+$ antibody-secreting cells (ASCs) through both T-cell–dependent (cytokine mediated) and T-cell–independent (Toll-like receptor (TLR) ligation) pathways. (2) Some IgA$^+$ ASC mis-home to the systemic compartment during lymphocyte trafficking. (3) Displaced IgA$^+$ ASCs take up residence in systemic sites and secrete normal 'mucosal-type' (poorly galactosylated and polymeric) IgA1 into the systemic circulation. (4) IgA1 secretion by displaced mucosal ASC is augmented by TLR ligation from mucosal-derived pathogen-associated molecular patterns, which have entered the systemic compartment. (5) IgA1 immune complexes form in the systemic circulation. Poorly galactosylated polymeric IgA1 molecules are the substrate for immune complex formation and combine with: (a) IgG and IgA autoantibodies reactive to exposed neoepitopes in the poorly galactosylated IgA1 hinge region; (b) antimicrobial antibodies specific for carbohydrate components of the microbial cell wall, which are cross-reactive with the poorly galactosylated IgA1 hinge region; (c) soluble CD89 that is shed from myeloid cells in response to polymeric IgA1 binding. (6) IgA1 immune complexes deposit in the mesangium through a combination of mesangial trapping and increased affinity of poorly galactosylated IgA1 for extracellular matrix components. Immune complex deposition triggers a series of downstream pathways leading to glomerular injury and tubulointerstitial scarring.

1.2.3.1 Genetic predisposition

Genome-wide association studies by Suzuki and Gharavi et al have identified five distinct susceptibility loci in the MHC locus on chromosome 6p21, the complement factor H locus on chromosome 1q32, and in a cluster of genes on chromosome 22q12 playing a potential role in antigen presentation, the formation of Gd-IgA1 and the cleaning of immune complexes by the alternative complement pathway (33, 35). The frequency of these genetic variants could to some extend explain the differences in prevalence rates of IgAN across different continental populations.
Recently, studies in Italy found a genetic predisposition to sporadic IgAN by polymorphisms in the genes C1GALT1 (coding for core-1-β,3-galactolsyltransferase, responsible for IgA galactosylation) and TLR4 (coding for Toll-like receptor 4) (36). In familial IgAN, genetic heterogeneity had been found with regard to the linkage to IGAN1 locus on chromosome 6 (37, 38). In minor patient cohorts, the susceptibility to IgAN has also been associated with variations in other genes such as those coding for transforming growth factor-beta 1, for angiotensin converting enzyme or, in males, for angiotensinogen (39-41).

1.2.3.2 Mucosal and systemic production of IgA1

Most of the systemic IgA in humans consists of the subtype 1. The monomeric form of IgA1 is released by the bone marrow, lymph nodes and spleen. Polymeric IgA1 is produced mainly by lymphocytes and plasma cells in the respiratory and gastrointestinal tracts. Abnormalities in the mucosal response to common microbial or alimental antigens may be involved in the synthesis of aberrantly glycosylated IgA1 as described below (42-44) and underglycosylated IgA1 is more prone to aggregate to polymeric forms (31, 45). The mucosal surface is the main site of the innate immunity, including the recognition of pathogen-associated molecular patterns (PAMPs) by macrophages, dendritic cells or leukocytes, preferably through binding to Toll-like receptors (46, 47). The innate immunity further links to the adaptive immunity by dendritic cell maturation and antigen-presentation to T-lymphocytes, leading to stimulation of antibody synthesis by B-cells. A dysregulation of both innate and adaptive immunity in IgAN may result in decreased mucosal antigen elimination and /or altered IgA1 synthesis promoting chronic inflammation (46). 'Homing' of CD4 positive T-cells and polymeric IgA1 (pIgA1) producing plasma cells from mucosal to systemic sites has been discussed as being contributory factors of the increased release of pIgA1 into the circulation in IgAN (48-51).

1.2.3.3 Aberrant glycosylation of IgA1

All immunoglobulins, including IgA1 are glycosylated. An altered pattern of the glycosylation of IgA1 has been recognized as a potential pathogenetic factor in IgAN for about two decades. It is driven by abnormalities of the expression and activity of the glycosyltransferases involved in the sequential post-translational modification of IgA1(52). The key feature of IgAN is the deficiency of galactose in the hinge region of the IgA1 heavy chains, exposing abbreviated glycans, composed of N-acetylgalactosamine (GalNAc) (Figure 2). These can be detected by ELISA methods using a GalNAc-specific lectin, *Helix aspera* agglutinin (53). Elevated circulating levels of Gd-IgA1 are found in patients with IgAN and in their relatives in comparison to in healthy controls (54). In patients with IgAN, the Gd-IgA1 is also detected in tonsillary B- cells (55-57) and in the immune-complexes deposited in the mesangium (58, 59).

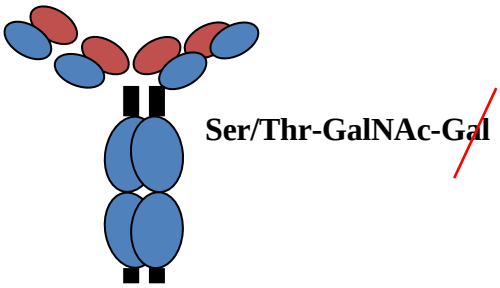


Figure 2. Galactose-deficient IgA1

1.2.3.4 Galactose-deficient IgA1- containing immune complexes and mesangial proliferation

Aberrantly glycosylated IgA1 can trigger an IgG autoimmune response forming IgG/IgA1 immune complexes (ICs) (52). 'Molecular mimicry' from environmental agents (such as ubiquitous bacterial and viral envelope proteins) is supposed to be involved in the autoantibody response to the neo-antigenic GalNac sites in the aberrant IgA1 (24). Serum values of IgG specific for Gd- IgA1 may serve as a more sensitive biomarker for IgAN than the levels of IgA1 itself (52, 53).

In IgAN, large IgG-IgA1 ICs are formed which likely escape clearance by hepatic receptors and enter the renal circulation where they pass through the endothelial fenestrae in the glomerular capillary wall and react with fibronectin, laminin and collagen within the mesangial matrix (31, 60, 61). It is not yet known what the pathogenetic role is of the transferrin receptor (CD71), which is expressed on the surface of proliferating human mesangial cells and can bind polymeric IgA1 as well as ICs containing Gd-IgA1. Binding of the ICs enhances the expression of the CD71 receptor in a positive feedback loop (62-64).

As shown by *in vitro* studies, the IgG-IgA1 immune complexes induce the mesangial cells to proliferate, secrete extracellular matrix components and release humoral factors such as tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), transforming growth factor beta (TGFβ), platelet derived growth factor (PDGF) and angiotensin II which further promote inflammation and enhanced glomerular permeability (65, 66). Complement activation via the alternative or mannose-binding lectin (MBL) pathway contributes to the renal damage (67-70).

The biologic activity of the galactose-deficient IgA1-containing ICs, assessed by their *in vitro* effect on mesangial cell-proliferation, increases in IgAN patients during episodes of macroscopic hematuria (71).

In serum, pIgA can also be found in ICs bound to a soluble form of the IgA-specific receptor CD89 (sCD89-pIgA ICs) that is normally expressed on the surface of monocytes, macrophages, neutrophils, eosinophils, dendritic cells and hepatic Kupffer cells but not mesangial cells and that belongs to a class of receptors that bind the Fc portion of immunoglobulins (72). Repeated samples in patients with IgAN, participating in the follow-up study at our department, showed consistently lower levels of sCD89-pIgA ICs, containing the 30kDa isoform of CD89, in patients with progressive disease compared to patients with more stable disease. A genetic single nucleotide polymorphism (SNP) associated with the lower levels of sCD89-pIgA ICs (73). This leads to the speculation that the binding of pIgA to sCD89 might protect from IgG-IgA1 immune- complex formation and secondary pathogenetic mechanisms (73, 74).

1.2.3.5 Podocyte injury in IgAN

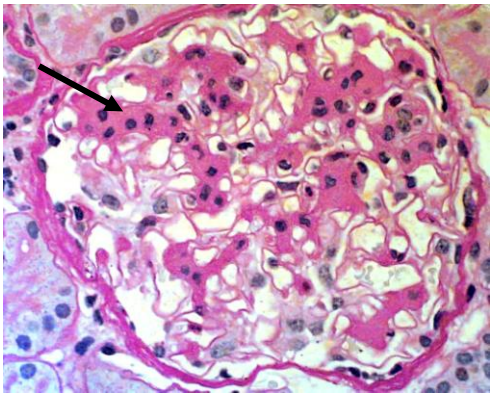
IgAN often shows lesions morphologically identical to those of focal segmental glomerulosclerosis (FSGS). According to recent studies, the segmental sclerotic lesions have features that suggest that they are due to primary podocyte injury (75, 76). It has been shown that aggregated IgA1 from patients with IgAN inhibits nephrin expression in podocytes (77, 78), mediated through TNFα and platelet-activating factor (PAF) (65, 79), and appears to produce podocyte apoptosis (66), as well as reduced adhesive capacity (80). Immunohistochemical studies in IgAN showed, in similarity to studies on FSGS, a focal loss of the podocyte markers synaptopodin, glomerular epithelial protein1 (GLEPP-1), nephrin and vascular endothelial growth factor (VEGF), particularly at sites of capsular adhesions without histological changes in the underlying glomerular tuft (76). The presence of free, podocyte marker bearing cells in Bowman´s space and the cumulative excretion of podocytes in the urine, linked to the prognosis of IgAN, all support the essential role of podocyte damage in IgA nephropathy (76, 81, 82).

1.2.4 Diagnosis and histopathologic classification

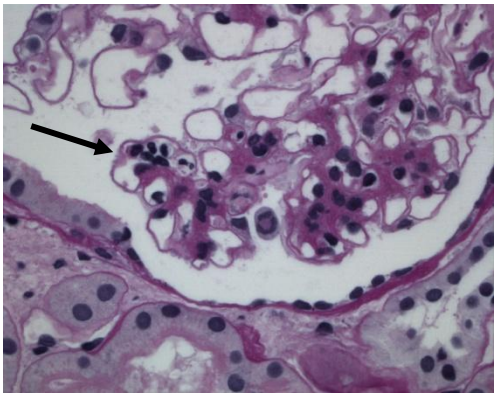
Until we have access to reliable noninvasive diagnostic tests, a diagnosis of IgAN requires a renal biopsy. Histopathological findings also contribute to the estimation of inflammatory activity and irreversible chronic changes. The value of this information for clinical decision making with respect to treatment is currently under intensive investigation.

Mesangial IgA deposits are the defining hallmark of the disease. IgG and IgM may accompany IgA but are found to a lesser extent. C3 deposition usually has the same distribution as IgA in the glomerulus and is also found in the wall of extraglomerular small vessels (83). The most common appearance in light microscopy is mesangial hypercellularity and accumulation of mesangial matrix. Crescentic changes may be superimposed on diffuse mesangial proliferative changes with or without associated segmental necrosis. Tubulointerstitial changes are similar to those seen in other forms of progressive GN, reflecting the final common pathway of renal parenchymal disease. Arteriolar vessel changes with hyaline atherosclerosis and media hypertrophy are frequently already found in early stages of CKD (84).

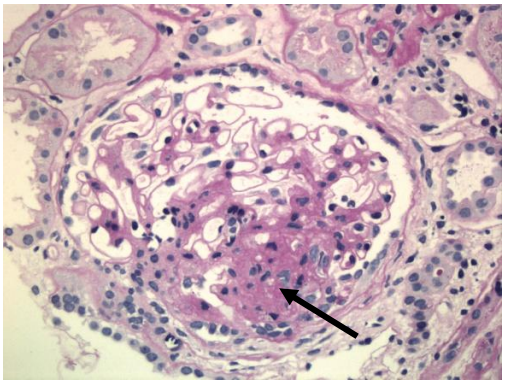
There has been a lack of international consensus in the validation of previous histopathological classifications (83, 85-88). In the new Oxford classification (OXFORD-MEST score) the International IgA Nephropathy Network has identified four histopathological lesions of independent prognostic importance; mesangial (M) and endocapillary (E) hypercellularity, segmental glomerulosclerosis (S), tubular atrophy and interstitial fibrosis (T). The results were analyzed using a systematic approach in order to develop a reproducible classification which could predict clinical outcome (89, 90). This classification has been validated in a number of different patient cohorts, adults and children (91-100), and some questions have been raised about the lack of cases with either milder or the most aggressive forms of the disease in the Oxford patient cohort, including cases with significant extracapillary proliferation. The large ongoing international multicenter VALIGA study, including more than 1200 patients with all spectrums of the disease, may lead to a new version of the Oxford classification. Figure 3 shows typical histopathological findings as described by the Oxford-MEST score:



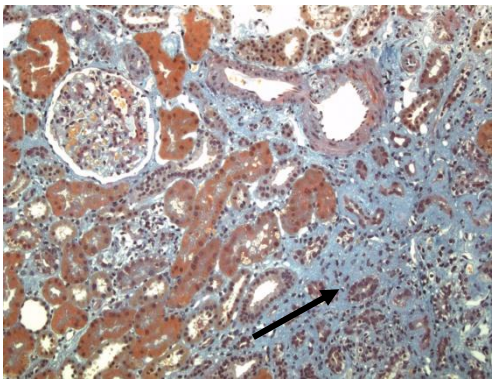
Mesangial proliferation (M)



Endocapillary proliferation (E)



Segmental glomerulosclerosis (S)



Tubular atrophy and interstitial fibrosis (T)

Figure 3: Histopathological lesions scored according to the Oxford-MEST classification

1.2.5 Natural history

Most published studies report on approximately 25 – 30% of patients requiring renal replacement therapy within 20 – 25 years of presentation and about 15% of patients within 10 years. Fewer than 10% of all patients with IgAN had complete resolution of urinary abnormalities, spontaneously or after treatment in most reports (27, 101-104). Repeated biopsy studies confirm that glomerular changes, including IgA deposits, can completely disappear spontaneously or after treatment in both native (105-107) or transplanted kidneys (108, 109). Contrary to what was previously thought, the disease seems to be as progressive in children as in adults (101, 110-112). Recurrence of IgAN after kidney transplantation occurs in about one third of patients, with large discrepancies between different series, mainly due to varying indications for graft biopsy and differences in follow-up duration as reviewed by Ponticelli 2010 (113). A clinical diagnosis of recurrent IgAN is made on average three years after transplantation. Recurrence is more frequent in younger patients and in those with a rapid progression of the original disease (109, 114, 115).

1.2.6 General mechanisms of renal disease progression

A number of sequential pathophysiological mechanisms have been identified as contributing to renal disease progression in general, presented by Harris and Neilson, Schlondorff and Sung et al (116-118) and they are summarized here:

1) Persistent **glomerular damage** due to inflammation or ischemia or a lower number of functioning nephrons from the outset, as seen in individuals with low birth weight.

2) Hyperfiltration in the remaining glomeruli with the loss of autoregulatory mechanisms. This results in the transfer of systemic hypertension to high intraglomerular pressure, contributing to glomerular and tubular hypertrophy. Epithelial cells (podocytes) can no longer cover the whole hypertrophied glomerulus and naked capillary loops lead to focal adherences to Bowman's capsule epithelium, more central parts of the capillary loop sclerose.

3) Hyperfiltration, also driven by metabolic factors like high glucose levels or a high protein load, promotes **proteinuria**. Hemodynamic changes contribute to a higher glomerular expression of cytokines and chemokines like interleukin-1 (IL-1), TNFα, monocyte chemoattractant protein-1 (MCP-1) or macrophage inhibitory factor (MIF), stimulating leukocyte-, trombocyte- and, further on, macrophage- and lymphocyte accumulation.

4) Proximal tubular cells are exposed to an amount of activating substances, not normally filtrated, like proinflammatory cytokines, chemokines, complement, ferritin, albumin-bound lipopolysaccharides, free fatty acids, immunoglobulins or growth factors such as insulin-like growth factor-1 (IGF-1) or TGFβ. Iron-complexes can contribute to oxidative damage. Also, there will be an overload of small proteins, normally reabsorbed by proximal tubular cells through endocytosis.

5) Growth factors and chemokines, filtrated and/or produced by activated tubular cells lead to the **recruitment of neutrophils and macrophages** that, in turn, produce cytokines and chemokines and induce **tubular apoptosis**. Fragments from filtrated proteins and necrotic cell components, which have been degraded by the tubular cells' proteasomes, are internalized by activated dendritic cells which, in turn, migrate to the renal lymph system and present those protein fragments as 'danger signals' **(DAMPS)** to cytotoxic T-cells through MHC klass II antigen presentation. The consequence is a successive **loss of self-tolerance**. Through these mechanisms, **tubular atrophy and interstitial inflammation** increase and nephritogenic T-cells accumulate. 'Pore forming proteins' (perforin, serin, esteras), produced by T-cells, lead to a higher permeability of the basement membrane. Similar effects are seen due to Toll-like receptor (TLR) activation through infections, metabolic factors, bacterial or viral ligands or free RNA from damaged cells. A cross-reaction with epitopes in the interstitium can also lead to immune-deposition. Accordingly, **complement is activated** both by the classical and the alternative pathway.

6) The increased stress on tubular cells through the overload of their endoplasmatic reticulum (**tubular ER stress**) and a structural change of the cells' cytoskeleton makes them transform to fibroblasts through so-called **epithelial-mesenchymal transition (EMT).** Those newly formed fibroblasts produce collagen molecules/matrix between the vasa recta and the tubular nephron

with negative impact on the survival conditions for the remaining tubular cells and ischemic damage to surrounding vessels. A disturbance of the balance between growth-factors and the proliferation of local fibroblasts can contribute to the damage, terminally leading to **non-cellular scar formation**.

Angiotensin II (Ang II) activation contributes to all these processes in a crucial way: by vasoconstriction of the efferent arteriole, Ang II activation leads to increased glomerular pressure, while vasoconstrictor effects on the afferent arteriole are counteracted by vasodilators such as nitric oxide (NO) and prostaglandins. Ang II effects also involve a reduction of the glomerular filters' size-selectivity and an increase of intracellular calcium in podocytes which contributes to changes in the cellular cytoskeleton.

Vascular endothelial cells produce cytokines, chemokines, growth factors and procoagulant factors in response to 'shear stress' which leads to trombocyte aggregation, and microtrombi, **endothelial degeneration** and hyaline depositions in the vascular wall as well as an inflammatory reaction with neutrophil-adhesion and macrophage-infiltration.

Important with regard to IgAN are the findings that **even mesangial cells,** after activation and proliferation, **can develop into a fibroblast-phenotype** with production of collagen, fibronectin, laminin, proteases and cytokines [IL-1, MCP-1, Regulation on Activation, Normal T Expressed and Secreted (RANTES), PDGF, TGFβ among others].

The general CKD progression theory, based on hemodynamic, proteinuric and inflammatory mechanisms secondary to nephron loss, **is also relevant in IgAN** as has been evaluated by a study by Bazzi et al (119). They found the ratio of the fractional excretion of IgG to the percentage of non-globally sclerotic glomeruli (FEIgG/SG) to be a reliable marker of tubular protein load in surviving glomeruli and a predictor of renal outcome.
Figure 4 provides an overview of the general mechanisms involved in renal disease progression and the focus of the studies included in this thesis.

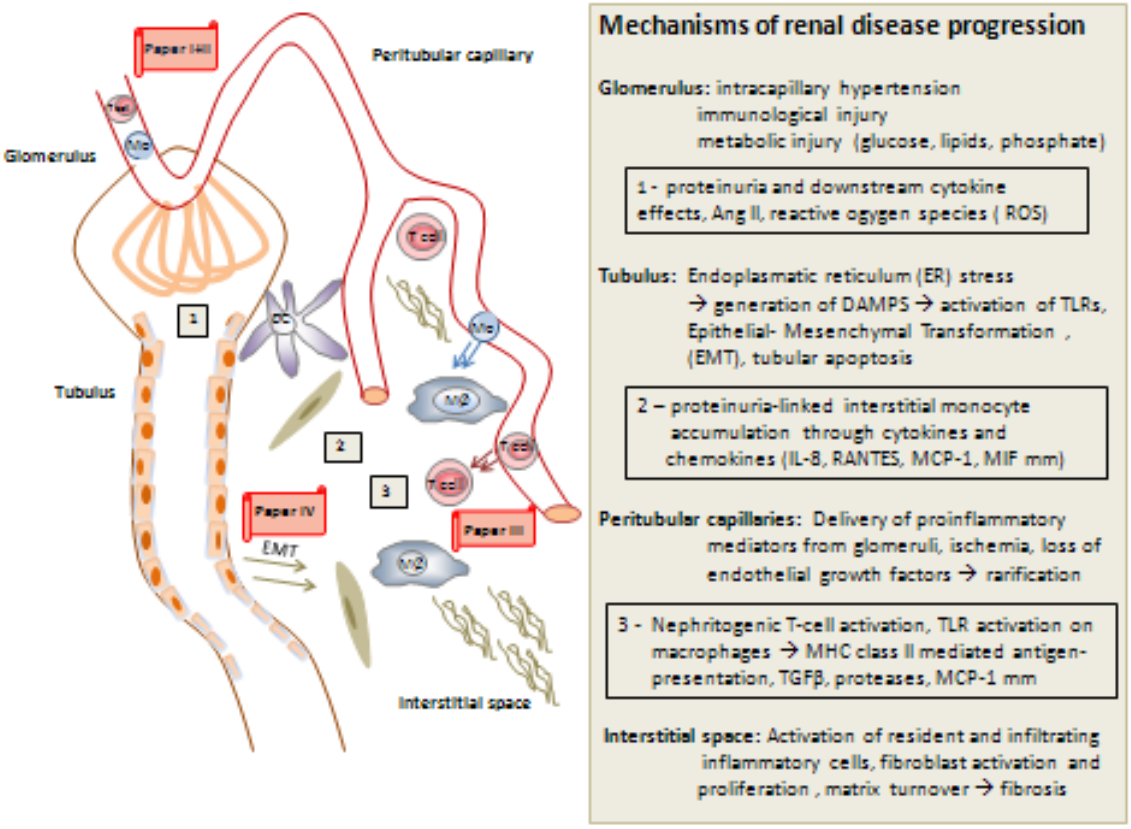


Figure 4: General mechanisms of renal disease progression and focus of the four studies included in the thesis: Paper I and II: metabolic injury, Paper III: activation of T-cells, Paper IV: disturbed tubular mechanisms. Mo, monocyte; MØ, macrophage; DC, dendritic cell; T-cell, T-lymphocyte; EMT, epithelial-mesenchymal transition.
Adapted from Schlondorff DO Kidney Int 2008.74:860-866 and Harris RC and Nelson EG, Ann Rev Med 2006.57:365-80

1.2.7 Risk markers for renal disease progression in IgAN

In an attempt to identify patients at higher risk for renal disease progression and therefore requiring more intensive follow-up and treatment, different scoring systems have been generated, as reviewed by Berthoux et al in 2011 (120) . Most of them include histopathological findings, making them more applicable at the time of diagnosis than during clinical follow-up. One of the difficulties in the prediction of prognosis in an individual patient with IgAN is the uncertainty about the disease course prior to the awareness of clinical symptoms leading to the diagnosis. This is illustrated by the findings that the impact of the major known risk factors, namely higher levels of proteinuria, serum-creatinine and blood pressure, varies depending on their onset, duration and possible relapse during the disease course (103, 104, 111, 121). The response of these parameters to therapy during follow-up is a strong indicator of renal outcome as is the presence or absence of advanced renal histopathological findings such as glomerular sclerosis and tubulo-interstitial fibrosis.

Smoking, hyperlipidemia, obesity, hyperinsulinemia and hyperuricemia have all been associated with renal disease progression in IgAN and other forms of chronic renal disease (122-128). Moderate alcohol intake may, however, be protective (129).
Little is known about the pathogenetic role and prognostic value of isolated inflammatory markers in blood or urine in IgAN. Some of the recent findings are presented in the 'General Discussion' part of this thesis. Markers of oxidative stress are discussed below. No differences between the sexes have been found with regard to renal outcome in IgAN which is in contrast to other forms of chronic GN (130).

Immunostaining of a subgroup of the renal biopsy specimens examined in the Oxford classification study cohort showed a possible impact of the distribution of IgA and IgG deposits in the glomerulus on the presence of proliferative lesions (131). The presence of capillary wall IgA deposits in addition to mesangial IgA deposits and the presence of IgG deposits were associated with a higher mesangial cellularity score and with the presence of endocapillary proliferation, supporting a causative role of these deposits in the development of proliferative changes. No significant association to renal outcome was found which might have been biased by treatment, as capillary wall IgA and the presence of IgG were associated with trends towards greater immunosuppression. Notably, in earlier studies, when RAAS blockade and immunosuppressive treatment were applied to a lesser extent, the extension of IgA deposition from the mesangial area to the peripheral capillary wall was found to be a significant adverse risk factor for renal disease progression (101). As a consequence, the location of IgA and IgG may be a factor to be taken into consideration in the prediction of prognosis and the decision on treatment but this has to be further validated.

1.2.8 IgAN associated to Henoch-Schönlein purpura

IgAN is closely associated with Henoch-Schönlein purpura (HSP), a small vessel vasculitis with deposition of IgA, predominantly affecting the skin, joints, gut and kidney (132). The nephritis of HSP (HSPN) is, like IgAN, characterized by mesangial deposition of galactose-deficient IgA1-containing immune complexes and is histologically indistinguishable from IgAN, though often showing more active proliferative and necrotic lesions (133, 134). Patients with HSPN have been documented in several pedigrees of related patients with IgAN (135, 136). Recently, a heritability of serum Gd-IgA1 has been shown in children with HSPN and children with IgAN (137) as has earlier been shown in adults with IgAN (54).
Due to the shared pathogenetic mechanisms, IgAN and HSPN are regarded by most authors as different clinical manifestations of one systemic disease. It is not uncommon that the same patient initially has clinical symptoms of Henoch-Schönlein purpura-associated nephritis, often with a good response to low doses of Prednisolone, if needed, but later on develops a more typical picture of chronic IgAN. The renal function can deteriorate many years after apparent remission which makes it mandatory to follow all patients for at least 5 – 10 years and to follow children with apparently benign HSPN or IgAN when they become adults (138, 139).
In most of the studies presented in this thesis both patients with IgAN and HSPN are included, as we presume that the mechanisms for chronic disease progression in these different conditions should be the same with regard to the factors investigated in our project.

1.2.9 Treatment of IgAN/HSPN

Initially, IgAN was regarded as a disease with a benign prognosis and the patients were often without treatment and were not regularly followed by a nephrologist. Since the middle of the 1980s the prognosis of IgAN has been reported to be worse than had been previously believed. After studies in 1994 (140) demonstrating the renoprotective effect of angiotensin converting enzyme inhibitors (**ACEIs**) and then of angiotensin receptor blockers (**ARBs**) in 2000 (141), these therapeutic agents began to be applied as the cornerstone of the treatment of patients with IgAN. Patients with hypertension, proteinuria more than 1g/day and reduced GFR are at high risk of progression and should be treated to a target blood pressure of 125/75 mmHg and a target proteinuria of < 0,5 g/ day with ACEIs and/or ARBs (28, 34, 111, 139). It is likely that the risk of proteinuria is a continuum which should be an argument to also treat patients with lower grades of persistent albuminuria, which is not as clearly evidenced in the literature (111). Uncontrolled hypertension has an additive effect with proteinuria in driving progression of the disease (120, 121).
There is no international consensus on the indications and dosages of **immunosuppressives**. The relative rareness of the disease and the slow progression rate are limiting factors to larger randomized controlled trials (RTCs) with sufficient follow-up periods. In a recent meta-analysis it was concluded that steroid therapy reduces proteinuria and the risk of ESRD (142).
Present recommendations are to add treatment with corticosteroids in patients with GFR > 30 – 50 ml/min/1.73m² when the level of proteinuria remains higher than 1 g/day or if the GFR declines by more than 30% during 3 – 6 months observation, despite optimal treatment with blockers of the renin-angiotensin-aldosterone system (RAAS) and optimal blood pressure control (139). Patients with lower GFR should receive immunosuppressive treatment only in the event of rapidly progressive glomerulonephritis due to extensive crescent formation. In these patients, cyclophosphamide should be used in combination with corticosteroids in the same way as in patients with other forms of crescentic glomerulonephritis, a recommendation that is mainly based on experience from case series (143). The optimal treatment in the presence of less than 50% crescents remains to be evaluated. Adequate supportive therapy may, to some extent, lead to the resolution of crescents (139). Patients with IgAN and coincident minimal change disease usually respond well to corticosteroid therapy (144).
Side-effects may be reduced by targeted corticosteroid treatment at the sites of mucosal B- cell induction which in a recent pilot study led to a significant reduction in urinary albumin excretion (145). Effectiveness with regard to renal outcome has to be proven by larger studies.
The evidence for the use of mycophenolate mofetil is still unclear and there may be racial differences in the response to this treatment (146). Azathioprine has not been shown to have any benefit for renal protection (147).
The STOP-IgAN trial, initiated by Floege and Eitner, will hopefully provide an answer to the question on risks and benefits associated with the addition of corticosteroids and cyclophosphamide to optimized supportive treatment. In this large German multicenter randomized controlled trial, optimized supportive therapy includes blood pressure control by ACEIs and/or ARBs, the use of statins and the reduction of dietary salt and protein intake.

Fish oil has beneficial anti-inflammatory and rheologic effects. It is safe and frequently prescribed in IgAN. However, according to a meta-analysis of available clinical studies the role of fish oil in renal protection is still inconclusive (148).

Tonsillectomy is widely applied in Japan in patients with IgAN but usually in combination with corticosteroids and its independent therapeutic efficacy has still to be proven (149-151). In European countries, tonsillectomy is currently only considered when there is a clear temporal relationship between tonsillitis episodes and relapses of macrohematuria or in the presence of other surgical indications. In a small Japanese study, tonsillectomy reduced proteinuria in transplanted patients with recurrent IgAN (152).

The risk of recurrent IgAN after kidney transplantation is not influenced by the immuno-suppressive regimen used, except for a protective effect of induction therapy with anti-thymocyte globulin, possibly related to augmented production of regulatory T cells (115, 153, 154). No specific therapy for recurrent IgAN is currently available.

Sufficient-sized studies on the treatment of Henoch-Schönlein purpura nephritis in adults are scanty and most data are retrieved from retrospective studies in adults and from experiences in children (155-161). Risk factors for progression were the same as for IgAN and a similar treatment approach is therefore recommended.

1.3 THE ROLE OF OXIDATIVE STRESS IN ATHERO- AND NEPHROSCLEROSIS

Markers of inflammation and oxidative stress have been shown to be significantly associated with cardiovascular disease and progression of renal disease and there is increasing evidence for a causative connection (162-167). Oxidative stress is an imbalance in naturally occurring cellular and molecular mechanisms with increased production of reactive oxygen species (ROS) and insufficient anti-oxidant ability of the cells. One of the mechanisms influencing how ROS contribute to cellular stress is the activation of Nuclear factor-kappa B (NFκB) that transcriptionally regulates genes involved in inflammation, immunity, cell proliferation, differentiation and apoptosis. The source of the increased amounts of oxidants in kidney disease can be both endogenous kidney cells and exogenous infiltrating cells (165, 167).
It has been suggested that activated monocytes play a pivotal role in the development of atherosclerosis. Monocyte activation by inflammatory molecules or microbes results in enhanced cell surface expression of adhesion molecules and cell infiltration of the vascular wall (162). Activation of monocytes also involves increased intracellular production of oxygen radicals which can be measured as monocyte respiratory burst. One of the effects of reactive oxygen metabolites in the circulation is the formation of oxidized low-density lipoprotein (ox-LDL), widely used as a marker of oxidative stress. Renal biopsies from patients with glomerular disease show accumulation of oxidized lipoproteins (168). In the kidney, perturbations of cellular oxidant handling promote cell apoptosis, senescence and fibrosis.

1.4 HYPERLIPIDEMIA AND STATIN TREATMENT IN RENAL DISEASE

Dyslipidemia in renal disease usually has the atherogenic profile of elevated low density lipoprotein (LDL), reduced high density lipoprotein (HDL) and high triglycerides (TGs). The loss of lipoprotein lipase due to increased glomerular permeability and the stimulation of hepatic lipoprotein synthesis secondary to albuminuria contribute to the hyperlipidemia in CKD.

Lipids may cause mesangial proliferation and bind to glycoaminoglycans in the glomerular basement membrane, increasing its permeability (169, 170). Filtrated LDL, especially in its oxidized form, is engulfed by foam cells, derived from macrophages, vascular smooth muscle cells and mesangial cells, which further contributes to the renal injury. (171). Numerous experimental and clinical studies have demonstrated that lipid derangements are an independent risk factor for the development of renal disease (126).
LDL is usually not directly measured but derived from the Friedewald formula. This formula cannot be used in the case of more pronounced hypertriglyceridemia, which is a common feature in patients with advanced kidney disease (172).

Apolipoproteins are critical in lipoprotein formation and clearance (173). ApoA-I and A-II are the major structural proteins of HDL particles (174). ApoB-100 is the major protein of all other lipoprotein particles including very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and lipoprotein a [Lp(a)] particles, thus reflecting the total number of these lipids. (175). Circulating VLDL particles are packed with triglycerides (TG) that are synthesized in the liver from endogenous fatty acids or dietary carbohydrates (176). VLDL particles with elevated TG are metabolized to small, dense LDL particles which are more atherogenic than normal size LDL particles (177). ApoB is considered to reflect the total number of potential atherogenic lipoprotein particles in plasma and it has been proposed that apoA-I corresponds to the level of potentially anti-atherogenic lipoproteins (178-180). Several studies have shown that the apoB/apoA-I ratio is superior in predicting cardiovascular outcome compared to total cholesterol levels, LDL-cholesterol, total/HDL-cholesterol ratio or triglycerides (179-186). Higher ApoB and lower ApoA-I values are frequently observed in CKD (187, 188).

A meta-analysis of 13 small studies in patients with renal disease demonstrated that the rate of decline in eGFR was lower in patients treated with statins than in those without this treatment (189). Statins exert immunomodulatory effects and may slow the progression of CKD by improving lipid profiles as well as by affecting inflammatory cell-signalling pathways (190, 191). Statins also protect against the oxidation of LDL (192). The anti-oxidant effects of statins enhance endothelial function and support normal vascular activity (193, 194). Furthermore, statins inhibit the activation of mesangial cells by ox-LDL and high glucose and may alter the inflammatory renal response to cytokines which may be augmented by dyslipidemia (195, 196).

1.5 T-LYMPHOCYTES IN IGAN

The systemic as well as the mucosal production of IgA appears to be controlled by T lymphocytes (197, 198). CD4 T lymphocytes (T helper cells, Th cells) play a critical role in the adaptive immune response and promote antibody production and class switching in B cells (199). Th cells can be divided into four main lineages, Th1, Th2, Th17 and Treg, characterized by the different main signature cytokines, Interferon gamma (IFNγ), interleukin-4 (IL-4), IL-17 and TGFβ, respectively.
IgAN has been described as a primarily Th2-dependent disease, although the pattern of peripheral T lymphocyte population, renal cytokine expression and phenotyping of intrarenal T-cells subsets in IgAN suggests that both Th1 and Th2 may be involved (200-204).

Interleukin-2 (IL-2) is produced by activated T cells and plays a pivotal role in the proliferation of T lymphocytes after antigenic stimulation. Upon activation, the T cell expresses high-affinity receptors for IL-2 (IL-2R), and subsequently, a soluble form of the IL-2R protein (sIL-2R, 45 kDa) is released. The release of sIl-2R appears to be a characteristic marker of continuous T lymphocyte activation and is attributed to playing a regulatory function during normal and abnormal cell growth and differentiation (205, 206). Previous experimental investigations and relatively small cross-sectional studies have demonstrated high production of IL-2 and IL-2R in IgAN (207-209). However, whether these factors are suitable as prognostic markers has not been validated in longitudinal studies.

1.6 KIDNEY-BONE AXIS AND THE ROLE OF FGF23

Fibroblast Growth Factor-23 (FGF23) is considered as a key player in chronic kidney disease-mineral and bone disorder (CKD-MBD), a syndrome defined by increased mortality, vascular calcification, fractures and biochemical abnormalities. FGF23 is a circulating hormone that promotes renal phosphate excretion and reduces the synthesis of active 1,25-dihydroxy vitamin D_3 by direct actions on the kidney (210, 211). In CKD, renal excretion of phosphate is compromised, leading to a compensatory rise in FGF23 level and development of secondary hyperparathyroidism (212, 213). Mild increments in FGF23 are commonly detected in CKD 2 - 3, whereas its circulatory levels are markedly elevated in ESRD (214-216).
High systemic levels of FGF23, both in CKD and in the general population, are linked to adverse outcomes including mortality and cardiovascular disease (CVD) (217-222). There is a debate ongoing as to whether FGF23 *per se* is detrimental to the cardiovascular system, or if FGF23 is merely a biomarker of multiple biochemical and metabolic derangements that parallel the progression of CKD. Recent experimental data favor the direct pathophysiological role of the FGF23 co-receptor Klotho in renal fibrosis and vascular calcification (223, 224), potentially explaining its relationship with CKD progression and CVD.

1.7 OBJECTIVES OF THE STUDIES

The overall aim of this thesis was to gain greater knowledge about mechanisms of renal disease progression in patients with a uniform diagnosis of chronic inflammatory kidney disease. We chose to study patients with IgAN as this is the most common form of glomerulonephritis in developed countries with a high percentage of relatively young individuals at risk of end stage renal disease and premature cardiovascular morbidity and mortality.

The specific objectives were to:

- Investigate peripheral blood monocytes as a source of oxygen radicals in chronic kidney disease and the *in vivo* effect of atorvastatin-treatment on this variable **(Study I)**.

- Evaluate the apolipoprotein B/apolipoprotein A-I ratio, shown to predict cardiovascular disease, as a potential risk marker for severe renal outcome **(Study II)**.

- Investigate the impact of increased T-lymphocyte activation, assessed by soluble Interleukin-2 receptor alpha levels, on renal disease progression and how it correlates to histopathological findings **(Study III)**.

- Investigate fibroblast growth factor 23 (FGF23), a regulator of mineral metabolism, as a risk marker for renal disease progression in IgAN and its correlation to albuminuria **(Study IV)**.

2 METHODS

2.1 IGAN/HSPN PATIENT COHORT

The patient population, that the four studies were based on, comprised all prevalent and incident adult patients with a renal biopsy-confirmed diagnosis of IgAN (with and without manifestations of HSP) who had been treated at the Departments of Nephrology at Karolinska University Hospital Solna between November 1994 and March 2009 and Danderyd University Hospital between February 2006 and May 2007 in Stockholm, Sweden (229 patients). The indication to perform a renal biopsy was the persistence of clinical symptoms suspicious of glomerulonephritis. Most of the patients had both hematuria and proteinuria with a proteinuria level greater than 0.5 g/ day but there were also 36 cases with less proteinuria on the date of renal biopsy, without ACEI/ARB treatment. Kidney biopsy is normally not performed at our department on patients presenting with isolated hematuria.

New patients included in the follow-up study at Karolinska University Hospital Solna after March 2009 and until September 2011 (n = 33) are integrated in the analyses described in the 'Additional Results' section. Only three patients declined to participate in the study.

As a rule, the patients had been followed at the outpatient clinics after the renal biopsy, irrespective of the severity of the renal disease. Of the 242 patients at Karolinska University Hospital Solna, included between 1994 and 2011, 27 patients (11%) were lost to follow-up as they had been referred to a general practitioner, moved away from the district or failed to come to the clinical visits. In one patient, follow-up was stopped after more than 10 years of complete remission. Thirty-one patients developed CKD stage 5 (13%) and 6 patients died (2%). Five patients were excluded during follow-up as they developed malignancies.

Figure 5 shows the annual number of incident and prevalent patients included in the project at Karolinska University Hospital Solna. On average, 13 patients per year have been included.

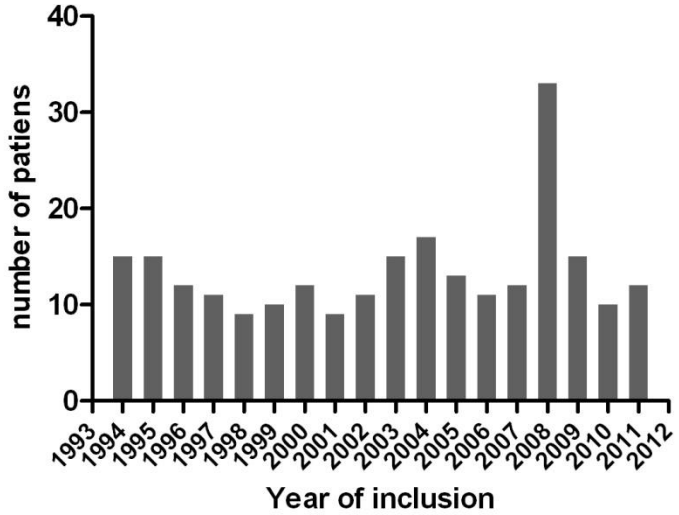


Fig 5: Number of patients included in the IgAN project by year at Karolinska University Hospital

The following clinical data are recorded in this project:

- Date (and age) of onset, diagnosis, follow-up evaluation
- Gender
- Ethnicity
- Inheritance (for IgAN, renal disease in general and ESRD, HT, cardiovascular disease, diabetes, rheumatologic disease)
- Concomitant disease/ smoking
- Disease manifestations at onset and reason for consultation
- Renal biopsy reports
- Debut of CKD stage 3 and 5, ≥50 % decrease of eGFR, start of RRT
- Debut of hypertension, CVD manifestations, date and cause of death
- Weight and height
- Medication at diagnosis, inclusion, follow-up visits
- History of tonsillectomy
- Annual blood pressure
- Annual routine laboratory results

In the studies presented in this thesis, we excluded patients with a concomitant diagnosis of malignancy (n = 2), diabetes (n = 7), inflammatory bowel disease (n = 1), rheumatoid arthritis (n = 4), severe ANCA negative systemic vasculitis (n = 1), antiphospholipid syndrome (n = 1), traumatic damage to the kidney (n = 1), nephropathia epidemica (n = 1) acute tubular necrosis (n = 2) or pregnancy (n = 1) or if they were ≥ 75 years old at the time of renal biopsy (n = 2). None of the patients had any liver disease or had started renal replacement therapy at baseline.

For follow-up analysis, only patients with an observation period of at least 1 year were included. In **study II,** only patients with a renal biopsy performed at Karolinska University Hospital Solna after 1992 were included and the patients had been followed until November 2006.
Study III and **IV** also included patients who had been diagnosed by renal biopsy before 1992 and patients who had been diagnosed at another Swedish nephrology unit and referred to our department due to movement to our district. The inclusion period was extended to March 2009 and patients had been followed until October 2010 (**study III**) or until May 2011 (**study IV**) respectively.
The subgroups of patients included in the different studies are described in the results sections for each study and are summarized in table 2.

2.2 HEALTHY CONTROL SUBJECTS

Eighty-four control subjects from a population-based cohort were obtained from the Swedish population register and were matched to the patient group in **study II and III** by gender and age. These individuals had been selected by exclusion of a diagnosis of renal disease, malignancy, diabetes mellitus, liver disease or chronic rheumatologic or inflammatory disease through the National patient register.

Table 2: Short summary of studies I – IV

	Aim	Number of study subjects	Inclusion criteria	Study design	Laboratory methods	Statistical analysis
I	Monocyte respiratory burst and effect of atorvastatin in IgAN	16 patients (16 controls in experimental study)	eGFR >20 ml /min/1.73m² not previously statin treated	Clinical trial and experimental study	FACS ELISA Routine biochemical analyses	Descriptive, ANOVA
II	Impact of apoB/ apo A-I on renal outcome in IgAN	70 patients 70 matched controls	CKD stages 1-3	Cross-sectional and prospective cohort study	Routine biochemical analyses	Descriptive, Kaplan-Meier estimates, Cox regression models
III	sIL-2Ra as a predictor of renal outcome in IgAN	194 patients 84 matched controls (179 patients in follow-up analysis)	CKD stages 1-5, (for follow-up study CKD stages 1-4) Not steroid-treated within 6 months	Cross-sectional and prospective cohort study	Luminex Routine biochemical analyses Histo – pathologic examination	Descriptive, Linear regression, Kaplan-Meier estimates, Cox regression models
IV	Relationship of FGF23 to albuminuria and renal outcome in IgAN	180 patients	CKD stages 1-4	Prospective cohort study	ELISA Routine biochemical analyses Histo-pathology	Descriptive, Linear regression, Kaplan-Meier estimates, Cox regression

Ethical approval

All studies were approved by the local ethics committee at the Karolinska University Hospital and, after 2004, the regional ethical review board, Stockholm, Sweden. Informed consent was obtained from all participants.

2.3 BLOOD SAMPLE AND CLINICAL DATA COLLECTION

At baseline, plasma and serum samples were spun and stored at -70° C. Clinical data and routine laboratory results were collected from the patients' records at the time of inclusion and once yearly thereafter. The healthy controls were asked to fill in a health questionnaire and a blood pressure test was performed in the sitting position after 5 minutes of rest.

2.4 ROUTINE LABORATORY AND CLINICAL MEASUREMENTS

Analyses of serum creatinine (reference < 100 µmol/l for men, < 90 µmol/l for women) were performed using routine methods. To correct for the different method-related reference values

during the period from January 1st 2001 until March 1st 2005, 5µmol/l were subtracted from the s-creatinine values obtained for standardization to isotope dilution mass spectrometry (IDMS), according to recommendations from the local laboratory. Calcium levels were corrected for s-albumin using the equation: albumin- corrected calcium = s-calcium + 0.02*(40 - s-albumin). For the assessment of albuminuria, patients had either provided a 24-hour urine sample or a morning urine sample for analysis of the urine-albumin/creatinine ratio (U-alb/cr, reference < 3.0 mg/mmol). From the latter samples, 24-hour albuminuria was calculated using the Cockroft Gault formula in accordance with Fournier (225) (U-alb/ 24 h = U-alb/cr*((140-age)*weight*1.77/ 1000)/1000 for men and U-alb/24h = U-alb/cr*((140-age)*weight*1.5/1000)/ 1000 for women). From the healthy subjects, a urine sample was obtained for analysis of the U-alb/cr ratio. U-alb/cr values of < 2 mg/mmol were defined as 1 mg/ mmol, for calculation of median values. Hematuria on dipstick was recorded in the range of 0 to 3+. Body mass index (BMI) was calculated by dividing a person's weight by the square of their height. Mean arterial blood pressure (MAP) was calculated as the sum of diastolic blood pressure and one third of the difference between systolic and diastolic blood pressure. GFR was estimated by the four parameter MDRD equation for s- creatinine values standardized to IDMS (226) and by the CKD-EPI equation (227).

2.5 FLOW CYTOMETRIC ANALYSIS

Flow cytometri or FACS (fluorescence-activated cell sorting) is a measurement of cell characteristics by laser-scanning while the suspended cells flow through the instrument. The refracted light (due to lens-like properties of the cell) and the reflected light (off the internal surfaces) provide information on the cells´ size and granularity, making it possible to differentiate between different white blood cells. The leukocytes are presented in a two-parameter scatter plot histogram by the computer output. Forward scatter (FSC), expressed on the y-axis, correlates to cell size and side scatter (SSC), expressed on the x-axis, correlates to the density of the cell, which depends on the number of cytoplasmic granules and membrane size. Fluochrome that reacts with components in the cell or fluochrome-marked antibodies can be used to increase the information about the cells. The fluochromes are excited by the laser and emit light of a different wavelength (i.e. color).

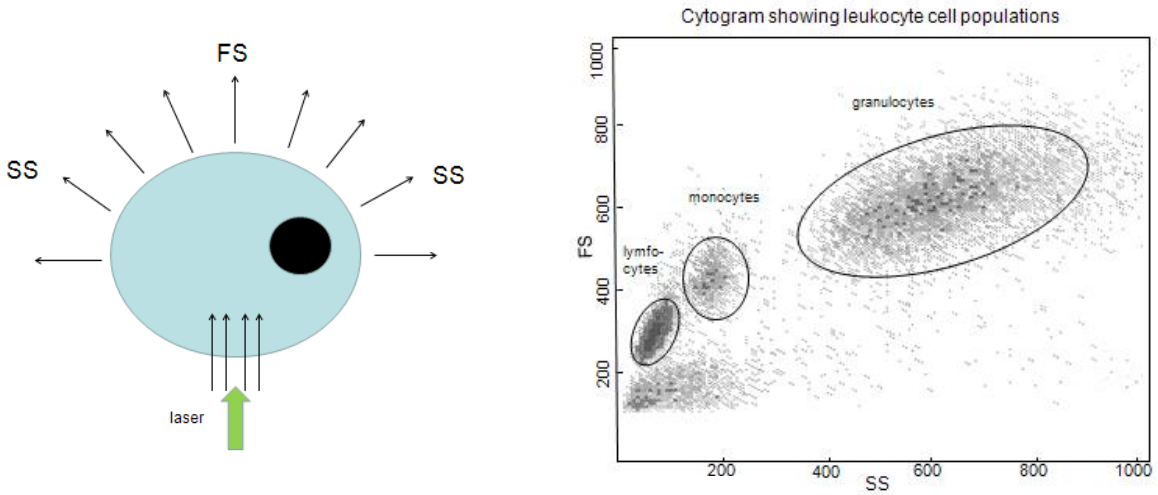


Figure 6. Principles of cell separation by cytometry

In **study I**, monocytes were selected by cytometry and their hydrogen peroxide formation (respiratory burst) was measured and quantified as mean fluorescence intensity (MFI). The analysis was performed in unstimulated cells and after stimulation of the cells with N-formylmethionyl leucyl phenylalanine (fMLP) or Phorbol-12-myristate-7-acetate (PMA), using the 2', 7'-dichlorofluorescein diacetate (DCFH-DA) method.

2.6 LUMINEX METHOD

The analysis of plasma sIL-2Ra concentrations (**study III**) was performed on the Luminex-100 system (Luminex Corporation) using Milliplex-kit (Millipore), according to the manufacturer's instructions. In short, microspheres are dyed to create distinct colors and coated with capture antibodies. The sample is added to the microspheres and the specific analyte will be captured. Fluorescent tagged detection antibody is added and a laser detects both the color of the microsphere and the tagged detection antibody. An advantage of this method is that only small sample amounts are needed. The lower detection limit was 3 pg/ml and a value of < 3 pg/ml was defined as 2 pg/ml for statistical analysis.

2.7 ANALYSIS OF OTHER BIOMARKERS

Enzyme-linked immunosorbent assays (ELISA, R&D Systems; Minneapolis, MN) for monocyte chemoattractant protein-1 (MCP-1) (minimal detectable dose, MDD, 5 pg/mL), serum intercellular adhesion molecule – 1 (sICAM-1) (MDD 0.35 ng/mL) , tumor necrosis factor receptor I and II (TNFR I and II) (MDD 0.6 pg/mL), and neutrophil gelatinase-associated lipocalin/ matrix metalloproteinase-9 complex (NGAL/MMP-9) (MDD 0.013 ng/mL) were done according to the instructions from the manufacturer. Oxidized LDL (ox-LDL) was analyzed by ELISA purchased from Mercodia AB (Uppsala, Sweden) **(study I and Additional results)**. C-terminal FGF23 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Immutopics, CA, USA). A radioimmunoassay (RIA) was used for the analysis of 25(OH) vitamin D (Immunodiagnostic-systems, Boldon, UK) (**study IV**).

2.8 DEFINITION OF RENAL OUTCOME

Disease progression was defined by different outcomes in the studies. **Study II**: entering ESRD (defined by CKD stage 5); **study III**: entering ESRD (CKD stage 5) or ≥ 50% reduction in eGFR or a 30% eGFR decline within 5 years of follow-up; **study IV**: entering ESRD (CKD stage 5) or ≥ 50% reduction in eGFR (endpoint 2A) and entering ESRD (CKD stage 5) or ≥ 25% reduction in eGFR within 10 years of follow-up (endpoint 2B). In studies III and IV, outcome was also defined by the annual decline in eGFR (eGFR slope) (**III, IV**) and by time-averaged albuminuria during follow-up (**IV**).
A composite endpoint was used in studies III and IV to reduce the number of misclassified patients due to a limited follow-up period.
In the '**Additional results'** section a new definition of endpoint was introduced, namely developing CKD stage 3b or higher (CKD stage 3b+) in accordance with the new KDIGO guidelines for stratification of CKD stages (eGFR < 45ml/min/ 1.73m²). The reasoning for this is discussed in the 'Findings and Implications' section.

2.9 EVALUATION OF HISTOPATHOLOGICAL FINDINGS

Only a few renal biopsies had both been performed close to baseline evaluation in the different studies and also been classified by the new Oxford-MEST classification. This classification is not applicable in patients with manifestations of HSP and biopsy specimens with less than eight non-globally sclerosed glomeruli. Comparisons between the levels of potential biomarkers and renal histopathological findings could thus only be made in small subgroups of patients (**studies III and IV**).
All available biopsy reports that were based on the Oxford-MEST classification were used in the analyses presented in the '**Additional results'** section.

2.10 STATISTICS (I-IV)

Descriptive statistics were used to characterize the study populations, presenting mean (± SD) for normally distributed and median (interquartile range) or median (range) for non-normally distributed values. Categorical variables were expressed as percentages. Spearman's rank correlation was used to analyze relationships between non-normally distributed values.
For cross-sectional analysis, comparisons of continuous variables between two groups were assessed using Student's unpaired t-test or Mann-Whitney U-test as appropriate. Comparisons between three groups were made using ANOVA test. Differences in proportions in different groups were compared by the Fisher's exact test.
Comparisons between repeated measurements in one group were made by Wilcoxon rank test.

Survival analyses were made using the Kaplan-Meier survival curve and log rank test for categorical variables and the Cox proportional hazards model for continuous variables. "Renal survival" was determined from the baseline examination, and patients were censored when not reaching the defined endpoint. Hazard ratios (HRs) for progression to the endpoint were determined by using univariate and multivariate Cox regression analysis and presented as HR and 95% confident intervals (CI).

Linear regression models were used to investigate the associations of biochemical and clinical variables. Risk ratios (RRs) and correlation coefficients (beta) were determined as RR (95% CI) or change per unit of the analyzed variable (95% CI) respectively.
Linear regression and the principal of least squares were also used to assess the annual eGFR slope by fitting a straight line through the calculated follow-up eGFR values.

Logistic regression was used to analyze the association of biochemical or clinical variables to a categorical outcome variable.

The two-sided p-value < 0.05 was considered to be statistically significant. Statistical evaluation was made by statistical software, STATISTICA 9 (StatSoft, Tulsa, UK) and STATA version 12 (Stata Corp, College station, TX, USA).

3 RESULTS AND DISCUSSION

3.1 PAPER I

Atorvastatin-induced modulation of monocyte respiratory burst *in vivo* in patients with IgA nephropathy: a chronic inflammatory kidney disease.

Respiratory burst of PMA-stimulated monocytes, measured by flow cytometry, was higher in 16 patients with IgAN (eGFR 48 ± 18 ml/min/1.73m^2) as compared to in healthy controls. After treatment of the patients with atorvastatin 20 mg/day during one month, there was a significant reduction of unstimulated, fMLP- and PMA- stimulated monocyte respiratory burst compared to baseline values (Figure 7). We observed no significant changes in ox-LDL, high sensitive CRP (hsCRP), MCP-1, ICAM-1, TNFR II and NGAL/MMP-9 and the urine-albumin/ creatinine ratio.

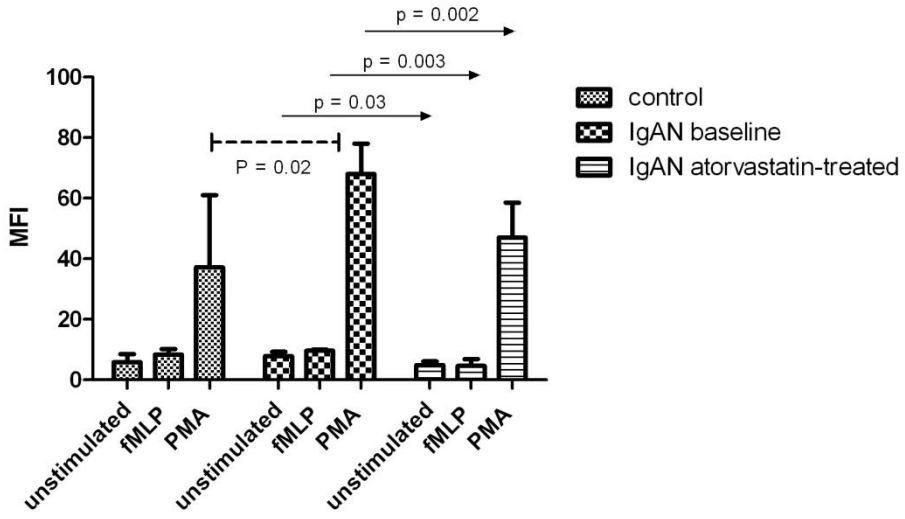


Figure 7. Monocyte respiratory burst in healthy control subjects and in 16 patients with IgAN before and after treatment with atorvastatin 20 mg/day during one month.
Respiratory burst is expressed as mean fluorescence intensity (MFI). The p-value is the result of Mann-Whitney-U test and Wilcoxon rank test respectively; fMLP, N- formyl- methionyl- leucyl-phenylanine; PMA, Phorbol 12-myristase 13-acetate.

Our results are in line with the study by Fortuno et al who found enhanced monocytic NADH oxidase-mediated superoxide anion production in response to PMA in patients with early stages of kidney disease (228). A metabolic hyperactivity of monocytes may contribute to the oxidative stress that has been linked to progressive renal disease as well as to the development of atherosclerosis. Circulating and filtrated proteins can be oxidized by ROS released from activated monocytes with the formation of advanced oxidation protein products (AOPP) (229). In IgAN, the level of AOPP was independently predictive of disease progression (230) . The AOPP level and the degree of Gd-IgA1 in combination correlated to proteinuria and the rate of renal function decline which indicates that oxidative stress may modulate the nephrotoxicity of aberrantly glycosylated IgA1 in IgAN (24, 231).

Statins block the synthesis of two isoprenoids that normally attach post-translationally to intracellular signaling proteins and the G-proteins (194). Through this pathway various statins have been shown to directly inhibit NADPH oxidase activity (192). This is supported by our findings that both fMLP-and PMA-stimulated respiratory burst of monocytes were inhibited by atorvastatin treatment, due to the fact that fMLP- stimulation is receptor-mediated whereas PMA directly acts intracellularly. Our study is small and should be confirmed by larger investigations but it is interesting as it shows an effect of atorvastatin on monocyte respiratory burst *in vivo*, supporting earlier *in vitro* findings in monocytes from healthy individuals (232, 233). Changes in the levels of ox-LDL after atorvastatin-treatment were of borderline significance in our study ($p = 0.06$)

We could not find any effect of atorvastatin-treatment on other inflammatory markers as could be expected according to earlier investigations (126). Presumably, an effect on systemic inflammation may be both time- and dose-dependent (234, 235) and can have been attenuated in our study by the RAAS blocking medications used in the majority of the patients.

3.2 PAPER II

Impact of apolipoprotein B/ apolipoprotein A-I ratio on renal outcome in IgA nephropathy.

The apoB/apoA-I ratio was significantly higher in 70 patients with IgAN and CKD stages 1 - 3 as compared to that in 70 age and gender matched healthy subjects ($p < 0.001$). ApoB was significantly higher in patients with IgAN than in healthy controls ($p < 0.001$) but there was no significant difference in apoA-I concentration.

Patients were followed over a period of up to 11 years (median 3.8 years). Threshold values for apolipoproteins were defined according to previously used and suggested values (236). Baseline apoB/apo A-I values greater than 0.9 for men and greater than 0.8 for women (= *high* apoB/apoA-I) were associated with the risk of developing ESRD (log rank test, $p = 0.02$, Figure 8). This association was independent of baseline eGFR [RR 6.50 (CI 95%, 1.20–35.12), $p = 0.03$] and independent of s-albumin [RR 5.63 (1.06-29.98), $p = 0.04$]. Seven patients had reached the endpoint, 5 belonging to the high apoB/apoA-I group.

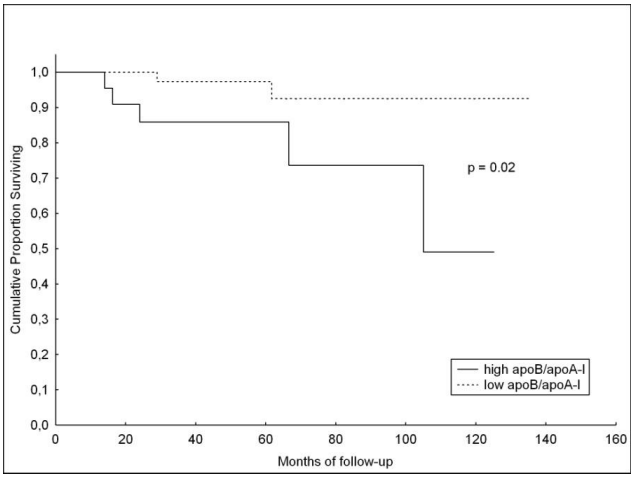


Figure 8: Survival rate curve for stable renal function in relation to the apoB/apoA-I ratio

The results of this prospective observational study show an increased risk of progression to ESRD in IgAN patients with a high apoB/apoA-I ratio which is a new finding. ApoB alone had borderline significance as a predictor for the renal outcome ($p = 0.06$).
In the patient group and in healthy subjects, the apoB/apoA-I ratio correlated significantly with other metabolic parameters at baseline such as age, BMI, serum creatinine and MAP and it correlated with hsCRP. There was no significant correlation between apoB/apoA-I ratio and baseline albuminuria which was unexpected but may be explained by the relatively low degree of median albuminuria at baseline in the study population. Only four patients had albuminuria in the nephrotic range (>3 g/24 hours) and all of them had a high apoB/apoA-I ratio. Two of these patients had well maintained renal function at baseline, received Prednisolone- and ACEI/ARB-treatment and had stable renal function at last follow-up. The other two patients were already in CKD stage 3 at baseline and progressed to ESRD within 16 months.
In accordance with earlier studies in patients with IgAN, eGFR, albuminuria and s-albumin at baseline, albuminuria at one year of follow-up and time-averaged MAP during follow-up were all significantly associated with more severe renal outcome (111, 237).

We could not confirm any association between BMI and renal outcome as has been shown in an earlier study in Chinese IgAN patients (127) even when we, in a secondary analysis, used the same outcome definition, which differed from ours (data not shown). Notably, the Chinese patient cohort was larger and also included some patients with concomitant diabetes, who were excluded in our study. The association between baseline hsCRP and renal outcome in IgAN has previously been investigated with conflicting results as discussed by Kaartinen et al (237). In our study, hsCRP was not associated with severe disease progression. The hsCRP levels in the patients were relatively low and were not significantly different from the levels in healthy controls which might have influenced our results.

3.3 PAPER III

Soluble interleukin-2 receptor alfa predicts renal outcome in IgA nephropathy.

Soluble IL2-Ra levels were significantly higher in 194 patients with IgAN (median age 39 years, 70% men) than in 84 age- and gender-matched controls ($p < 0.001$). One hundred seventy nine patients with CKD stages 1 – 4 at baseline were followed for up to 16 years (median 52 months, range 12 – 188). Soluble IL-2Ra levels in the upper third tertile predicted a severe renal outcome, defined as development of CKD stage 5, a 50% decline in GFR during the follow-up period or a 30% GFR decline within 5 years of follow-up, even after adjustment for age and the main clinical risk factors: eGFR at baseline, time-averaged (TA) albuminuria and TA MAP during follow-up (RR 5.67 (2.17-14.84), $p < 0.001$). Age, BMI and MAP at baseline were not significantly associated with renal outcome.
In the analysis presented in the paper we chose to stratify sIL-2Ra by tertiles due to its non-normal distribution. Actually, sIL-2Ra was clearly log-normally distributed and we therefore, in a secondary analysis, also examined the association of natural log-transformed sIL-2Ra to renal outcome. As shown in table 3, log sIL-2Ra was a significant predictor of severe renal outcome

also after adjustment for the established risk factors baseline eGFR, TA albuminuria and TA MAP. Further adjustment for age, which was not predictive in crude analysis, did not change the results.

Table 3. Unadjusted and adjusted risk estimates by Cox´s proportional hazard models for progression to the combined endpoint in the follow-up IgAN patient cohort (n = 179)

Variables	**HR**	**95% CI**	**p**
Log sIL-2Ra, crude analysis - adjusted for eGFR [a] , TA albuminuria and TA MAP	1.80 1.79	1.34 – 2.43 1.25 – 2.57	<0.001 0.002
eGFR [a]	0.57	0.47-0.69	<0.001
Albuminuria at baseline	1.59	1.34-1.89	<0.001
TA albuminuria	2.42	1.90-3.08	<0.001
TA MAP	1.09	1.05-1.13	<0.001

Albuminuria ,urine-albumin per 24 hours (g/d); TA, time-averaged during follow-up; MAP, mean arterial pressure (mmHg); [a] stratified by 10 ml/min/1.73m²

According to the Oxford classification, the presence of more than 25% tubular atrophy/ interstitial fibrosis (T1-2) was associated with higher sIL-2Ra levels, after adjustment for s-creatinine levels. Table 4 shows further associations found in the subgroup of patients with a renal biopsy classified by Oxford-MEST score and performed close to baseline analysis.

Table 4. Summary of p- values for differences in laboratory results and clinical findings at baseline with respect to Oxford classification scores in renal biopsies performed within 4 months (n = 24)

	M0:M1 (n= 20:4)	***E0:E1 (n = 20:4)***	***S0:S1 (n=19:5)***	***T0: T1-2 (n=17:7)***
sIL-2Ra	0.35	0.16	1.00	**0.013**
s- creatinine	0.35	0.18	0.33	**0.003**
Degree of hematuria	**0.029**	**0.029**	0.16	0.15
Albuminuria	0.157	0.210	**0.044**	0.099
MAP	0.115	0.157	**0.019**	0.664
BMI	0.682	0.494	0.230	0.153
Time from onset to biopsy	0.682	0.737	0.891	**0.034**

P-values (two sided) are results of Mann Whitney U- test, $p < 0.05$ in bold. sIL-2Ra, soluble Interleukin 2 receptor alfa; degree of haematuria on dipstick (0,1+,2+,3+); MAP, mean arterial pressure; BMI, body mass index.

In the 265 patients included in the Oxford classification study, proliferative changes and segmental glomerulosclerosis were strongly associated with proteinuria at the time of biopsy. Segmental glomerulosclerosis also was associated with higher initial MAP as in our patient group. Tubulointerstitial changes were associated with a reduced initial eGFR, higher initial MAP and proteinuria. The correlation of the different factors included in the MEST-score with the degree of hematuria is not described in the original paper by Cattran et al or the validation studies of the Oxford classification referred to in this thesis (89, 96-98).

This is the first report of an association of sIL-2Ra levels with renal disease progression, independent of other well-established clinical risk factors. The findings of increased levels of sIL-2Ra in IgAN are in agreement with cross-sectional and experimental studies performed by other groups (207-209, 238). A disturbed mucosal immune response to environmental factors such as microbial or food antigens may lead to activation of the innate immune system, triggering of inflammatory transcription factors and the generation of mediators that activate the adaptive immune system constituted by T and B cells (46). T-cell activation also has been shown to contribute to the altered glycosylation of IgA1, which plays a predominant role in the pathogenesis of IgAN (30, 239-241).
Moreover, infiltrating T lymphocytes in the peritubular space sustain interstitial inflammation thus further promoting the development of interstitial fibrosis and tubular atrophy as described in figure 4 (242-244).
This study provides further support for the view that IgAN is a T-cell driven disease and that increased T-cell activation in progressive disease may be a suitable target of therapy.

3.4 PAPER IV

FGF23, albuminuria and disease progression in patients with chronic IgA nephropathy.

FGF23 was measured at baseline in 180 patients with IgAN in CKD stages 1 – 4 and without any concomitant disease. FGF23 values (median 18.5 RU/mL, range 5.4 – 138.7 RU/mL) significantly correlated with albuminuria, serum albumin, eGFR, calcium, parathyroid hormone (PTH), MAP, BMI and the number of antihypertensive drugs used at baseline. There was no significant association between FGF23 and phosphate, 25-hydroxy-vitamin D (25(OH) vitamin D) or age. Patients who were treated with ACEIs and/or ARBs had higher FGF23 levels compared to those without this treatment.
The patients had been followed for a median of 55 months (range 12 – 177 months). Time-averaged albuminuria during follow-up was 0.18 g/24 hours (range 0 – 7.4 g/24 hours) and the annual loss of eGFR was 0.8 ml/min/1.73m² (range -8.4 to 32.3 ml/min/1.73m²). In multivariate regression models, log-transformed FGF23 levels at baseline correlated to time-averaged albuminuria and to the annual decline of eGFR during follow-up, independent of other metabolic factors and independent of baseline albuminuria, eGFR and the treatment with ACEIs or ARBs (tables 5a and b).

Table 5. Multivariate regression model predicting a) time-averaged albuminuria (g/24 hours) and b) the annual loss of eGFR (ml/min/1.73m²)

a)

Variable	Change in time-averaged albuminuria	P for Trend
per increase in log FGF23 (RU/ml)	0.26 (0.05 - 0.47)	0.02
per 10 ml/min/1.73m² of eGFR	-0.05 (-0.10 to -0.005)	0.03
per 0.1 mmol/L of calcium	-0.24 (-0.35 to -0.13)	<0.001
per 1 g/24 hours of albuminuria	0.45 (0.34 - 0.57)	<0.001

b)

Variable	Change in annual eGFR loss	P for trend
per increase in log FGF23 (RU/ml)	1.50 (0.40 - 2.60)	0.008
per 0.1 mmol/L of calcium	-1.04 (-1.66 to -0.41)	0.001
per 1 g/24 hours of albuminuria	1.46 (0.85 - 2.07)	< 0.001

In a stepwise regression model log FGF23, age, gender, s-albumin, baseline albuminuria, baseline eGFR, calcium, phosphate, PTH, 25(OH) vitamin D, mean arterial pressure, body mass index and the use of ACEIs and/or ARBs were included. Log FGF23, calcium and albuminuria were retained as significant (adjusted $R^2 = 0.44$, $p < 0.001$ for the whole model in a) and $R^2 = 0.22$, $p < 0.001$ for the whole model in b). The tables depict the regression coefficients (95% confidence interval) adjusted for the listed variables. FGF23, fibroblast growth factor 23; RU, relative units.

For survival analysis, renal endpoints were defined as either A) entering ESRD (CKD stage 5) or ≥ 50% reduction in eGFR or B) entering ESRD or ≥ 25% reduction in eGFR during 10 years. Using the MDRD eGFR equation, 15 patients (8%) were defined as progressors according to endpoint A and 34 patients (19%) reached endpoint B. Using the CKD-EPI eGFR equation a total of 17 patients (9%) reaching endpoint A and 39 patients (22%) reaching endpoint B were identified. LogFGF23 was significantly associated with renal outcome in all these models, independent of other markers of mineral metabolism and independent of eGFR and MAP or baseline albuminuria. When adjusted for all the factors included in the multivariable regression analysis described above, log FGF23 remained to be a predictor for outcome B, independent of whether eGFR was estimated by the MDRD or the CKD-EPI equation, but the association to endpoint A was no longer significant.

Inspired by the prognosis model by Berthoux et al (120), we calculated a risk score that could be used during clinical follow-up, including the presence of hypertension, albuminuria ≥ 1g/day and FGF23 levels > 23 RU/mL. Ten of 22 patients with all three risk factors at baseline (45%) reached endpoint A (HR compared with 0-2 risk factors: 20.53 (6.43 – 65.54), $p < 0.001$) and 15 of these 22 patients (68%) reached endpoint B (HR compared with 0-2 risk factors: 9.71 (4.86 – 19.40), $p < 0.001$).

In summary, we report on novel associations between FGF23 and albuminuria across the spectrum of CKD stages 1 – 4 and a predictive value of baseline FGF23 levels for both the persistence of albuminuria during follow-up and for renal outcome in IgAN.
The value of FGF23 as a potential biomarker for cardiovascular pathology has been supported by a number of recent studies where FGF23 was associated with endothelial dysfunction, atherosclerosis and left ventricular hypertrophy (217, 218, 221, 245). An association with renal disease progression has earlier been found in a heterogenous CKD population (246) and in transplant recipients (247) but not in patients with a single underlying renal diagnosis. It is assumed that FGF23 may reflect the over-all risk exposure to the factors that determine its expression, including hyperphosphatemia, dietary phosphate load, the degree of secondary hyperparathyreoidism, vitamin D levels, a decrease in its cofactor Klotho and RAAS activation. At higher concentrations, FGF23 may also exert non-specific and Klotho-independent effects

(248, 249) which could directly contribute to glomerular damage, despite the localization of Klotho being restricted to the tubuli. This has still to be proven. Alternatively, FGF23 levels might reflect the vascular status that secondarily aggravates a decline in renal function and/or proteinuria.
In our study, we did not find any association of vitamin D levels with albuminuria or renal disease progression which could have been suggested by experimental studies showing that vitamin D treatment can suppress the RAAS and other pathological mechanisms involved in the progression of proteinuric renal disease (250-252). Treatment modalities in our study, with the majority of patients treated with ACEIs or ARBs at baseline and 3% on calcitriol supplementation, may have influenced our results.
Phosphate levels were predictive of renal disease progression to either of the endpoints in crude analysis but not in multivariable models.

3.5 ADDITIONAL RESULTS

3.5.1 Soluble TNF receptors, albuminuria and renal outcome

TNFα is produced by a variety of cells, including monocytes, macrophages, adipocytes, B- cells, T-cells, dendritic cells and mast cells. As a central mediator, it is involved in the innate and adaptive immune responses. TNFα exerts its actions through two structurally distinct receptors, TNFR I and II, found on nearly all cell surfaces, and induces the expression of adhesion molecules, chemokines and apoptosis in susceptible cells (253, 254). Both receptors are shed into the circulation as a response to inflammatory signals. Soluble TNFRs are more easily measured than TNFα since they occur in the circulation at 100 –1000 times higher concentrations. The role of sTNFRs can be that of natural antagonists, neutralizing the effect of TNFα, or that of ligand passers that keep TNFα in a circulating reservoir, thus protecting it from degradation and, over time, possibly enhancing its effects. In experimental models, TNFα causes direct glomerular injury (255, 256) and in diabetic patients, TNFα levels have been associated with the severity of proteinuria (257). TNFα levels in the supernatant from peripheral blood lymphocytes were higher in patients with IgAN than in healthy controls (258). It has also been shown that polymeric IgA stimulates mesangial TNFα production (259) and that mesangial derived TNFα in IgAN is a mediator for the mesangial-tubular and mesangial-podocytic communication (65, 260). Moreover, increased TNFα levels have been associated with cardiovascular risk (261) and are known to influence lipid metabolism (262). In 43 patients from our study population, examined within 6 months of renal biopsy, (eGFR 56 ± 26 ml/min/1.73m²) we had earlier found a close linear correlation between levels of soluble tumor necrosis factor receptors I and II (TNFR I and II) and renal function with a correlation coefficient as high as $r = 0.94$, $p < 0.001$ for sTNFRI vs creatinine and $r = 0.89$, $p < 0.001$ for sTNFR II vs creatinine (presented as a poster at 11th International Symposium on IgAN in Tokyo 2006) which is even higher than the associations found in other CKD patient cohorts (263, 264).

We now repeated the measurement of TNFR levels in the 179 patients included in the follow-up analysis in **study III.** Similar to s- creatinine and sIL2-Ra, the levels of sTNFR I and II showed a log-normal distribution whereas baseline eGFR was normally distributed. Figure 9 shows a strong linear correlation of log TNFRI and II to baseline eGFR ($r = -0.73$, $p < 0.001$ and $r =$

-0.66, $p < 0.001$ respectively). The correlation of log sIL-2Ra to baseline eGFR was also significant but weaker ($r = -0.22$, $p = 0.003$).

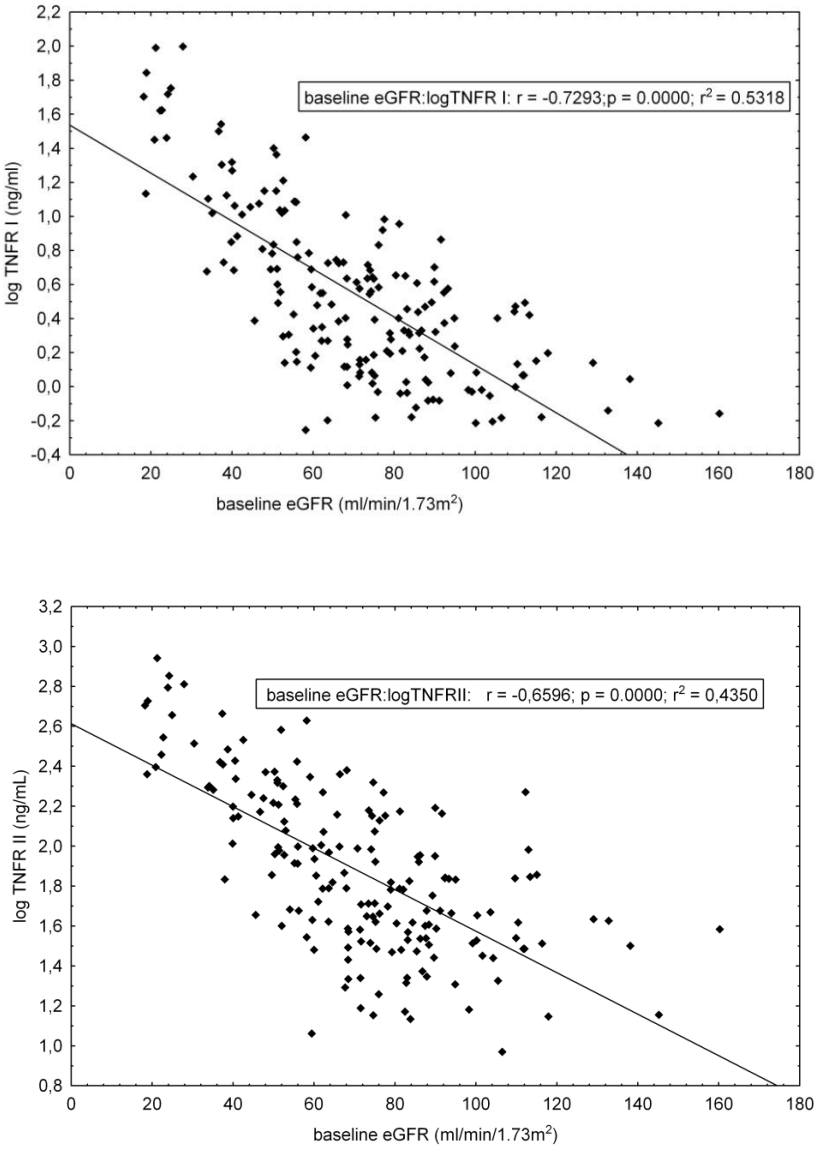


Figure 9. Correlation of logTNFR I and logTNFR II to baseline eGFR

In multivariable regression analysis, adjusted for age, baseline eGFR, BMI, MAP and the use of ACEIs or ARBs, logTNFR I and log TNFR II independently correlated to baseline albuminuria [**Regression coefficient for change in albuminuria (g/24 hours) per increase in log TNFRI**: $\beta = 1.03$ (0.56 – 1.50), $p < 0.001$ and **per increase in logTNFRII** : $\beta = 1.06$ (0.55 – 1.57), $p < 0.001$)]

Log TNFR I and II also correlated to logIL-2Ra and the association was independent of baseline eGFR and baseline albuminuria [**Regression coefficient for change in log IL-2Ra per increase in logTNFR I:** $\beta = 2.08$ (1.23 – 2.93), $p < 0.001$ and **per increase in logTNFR II:** $\beta = 2.11$ (1.18 – 3.04), $p < 0.001$].

In survival analysis, baseline logTNFR I and logTNFR II, similar to logIL-2Ra, were significantly associated with the combined renal endpoint as defined in **paper III**, after adjustments for baseline eGFR, TA albuminuria and TA MAP (Table 6).

Table 6. Risk estimates by Cox's proportional hazard models for progression to the combined renal endpoint, adjusted for baseline eGFR, TA albuminuria and TA MAP.

Variables	HR	95% CI	P
Log TNFR I (ng/mL)	8.37	1.83 – 38.30	0.006
Log TNFR II (ng/mL)	42.03	7.09 – 249.16	< 0.001

The correlation of log-transformed TNFR levels to eGFR in our study population was in concordance with the findings in heterogenous CKD patient cohorts (263, 265, 266) and in type 2 diabetic patients without proteinuria (267). In the latter study, diabetic patients with both TNFR I and TNFR II levels above median values (defined as high level) had a greater decline in renal function compared to those with a high level of only one receptor or a low level of both receptors.

The predictive value of TNFR I and II for renal outcome in IgAN, independent of other strong progression risk factors is a new finding. This is in line with the hypothesis that plasma TNFR levels in patients with IgAN, and possibly also in patients with CKD due to other causes, not only are elevated due to retention when the glomerular filtration ratio falls but may also reflect an 'inflamed' state that increases in lower CKD stages and further promotes renal disease progression. In a post hoc analysis of the CARE study (268), patients with sTNFR II levels in the upper tertile, similar to patients with CRP levels in the upper tertile, had a more rapid renal function loss compared to those with sTNFR II- or CRP levels in the lowest tertiles. Interestingly, despite pravastatin-treatment not being associated to renal function loss in the whole study cohort, subjects with both CRP and sTNFR II in the highest tertile appeared to derive more renal benefit from pravastatin than those without. In these 'inflamed' patients the renal function loss in patients receiving pravastatin was 0.8 mL/min/1.73 m² slower than in those receiving placebo (269).

3.5.2 Patients with IgAN and HSPN at one single center

The initial reasons for consultation of a doctor in the 242 patients who have been treated at Karolinska University Hospital Solna represented common clinical presentations of IgAN as shown in figure 10. All patients with renal findings of IgAN who had typical skin manifestations of HSP were defined as having HSPN. Immunofluorescence staining of skin biopsies for the detection of IgA deposits was not routinely performed. Similarities and differences in hereditary and clinical parameters between patients with IgAN and patients with HSPN, according to this definition, are presented in table 7.
Ten patients in the IgAN group had malignant hypertension at presentation.

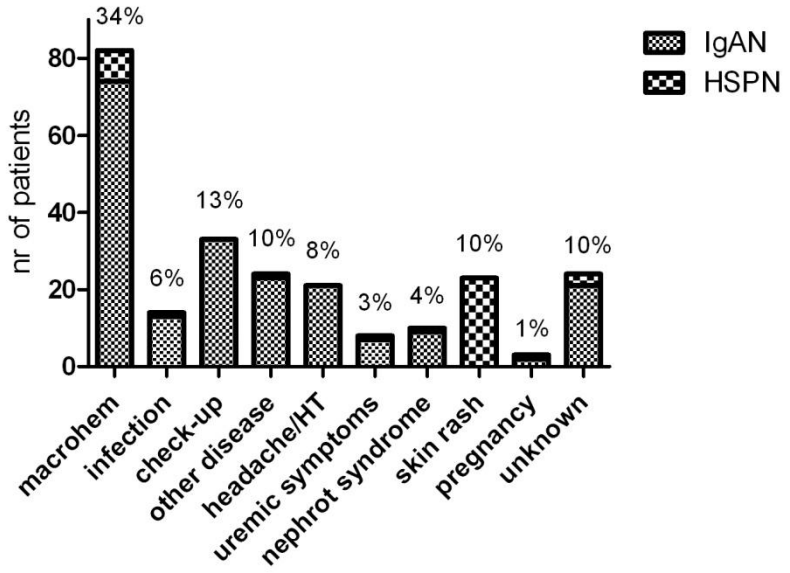


Figure 10. Reasons for consultation in 242 patients at Karolinska University Hospital Solna.

Table 7. Demographics of 222 patients with IgAN without and with manifestations of HSP (= HSPN) (17 IgAN patients and 3 HSPN patients excluded due to concomitant disease)

Characteristics	IgAN	HSPN	p- value
No of patients	N = 188	N = 34	
Female	30%	41%	0.23
Caucasian	94%	94%	
Biopsy at Karolinska	87%	91%	
Child at onset / at diagnosis	17% / 7%	21% / 9%	0.72
Time from onset to diagnosis (months)	1.3 (0 - 36)	0.4 (0 – 24)	**0.03**
Age at diagnosis (years)	33 (9 – 77)	38 (11 – 78)	0.08
Family history of renal disease[a] / ESRD Family history of HT	**16% / 4%** **39%**	**12% / 6%** **35%**	**0.61 / 0.39** **0.54**
Follow-up from diagnosis (years)	6.3 (0 - 36.7)	5.9 (0 – 15.6)	0.58
Treated with corticosteroids / + Cyclophosphamide	**13%** / 0.5%	**56%** / 12%	< **0.001**
Treatment at last follow-up - ACEI/ARB - Statin - Fish oil - Vitamin D	 78% 23% 13% 19%	 50% 21% 6% 6%	 **0.003** 1.0 0.38 0.12
Tonsillectomy	4%	6%	0.65
Developed hypertension /CVD	**73%** / 5%	**65%** / 9%	**0.39/ 0.40**
Developed ESRD (CKD stage 5)	14%	9%	0.59
Developed CKD stage 3b+	33%	15%	0.08
Developed CKD stage 3b+ before age 45	**N = 50/62 (81%)**	**N = 4/5 (80%)**	
Albuminuria at last FU < 0.2g/day	63%	79%	0.09
Death	2%	3%	

[a] Three percent of IgAN patients and none of HSPN patients had a family history of biopsy-confirmed IgAN/HSPN.
Numeric values are median (range); p-values were calculated by Fishers' exact t-test or Mann-Whitney-U test

For this analysis, patients had been excluded in the presence of concomitant diseases of either diabetes (n = 7), malignancy (n = 2), chronic rheumatologic (n = 5) or chronic inflammatory bowel disease (n = 2), traumatic damage to the kidney (n = 1), acute tubular necrosis in hypersensitivity reaction to an antibiotic (n = 1), nephropatia epidemica (n = 1) or organ damage secondary to alcoholism (n = 1). An additional endpoint was defined as CKD stage 3b+ (eGFR < 45ml/min/1.73m²) and we found that more than 80% of the patients who had developed CKD stage 3b+ reached this endpoint before the age of 45 which implies a high risk of the need of RRT during working age and also an enhanced risk of premature cardiovascular disease**.**

Comparing patients with IgAN with and without manifestations of HSP, there were no significant differences in sex, onset or diagnosis in childhood, family history of renal disease/ ESRD, the percentage of patients that had developed hypertension at onset or during follow-up or the percentage of patients that had developed ESRD.

On the other hand, patients with HSPN had been diagnosed earlier after the onset of clinical symptoms and had received immunosuppressive treatment to a higher extent. Patients with HSPN were to a lesser degree on treatment with ACEIs/ARBs at last follow-up which might be explained by the fact that more patients with HSPN compared to patients with IgAN tended to be in clinical remission with regard to albuminuria at that time. Also there was a trend towards fewer patients with HSPN, compared to patients with IgAN, developing CKD stage 3b+. Thirty-nine percent of the IgAN patients who developed CKD stage 3b+ had been diagnosed before 1998 when the current recommendations for treatment with ACEIs/ ARBs and immunosuppressives had not yet been established.

The discrimination between IgAN and HSPN is complicated by the fact that some patients may have a clinical picture of HSPN in childhood which with progressing age acquires all the features of chronic IgAN. This was also the case in the patient population presented here.

3.5.3 Can we predict renal outcome in individual patients?

Clinical data at time of diagnosis have been used in most of the proposed prognostic models for renal outcome in IgAN (120, 270-273). Few of them have been validated in independent patient cohorts and they have not been widely adopted.

The scoring system for ESRD risk prediction in groups of patients, developed by Goto et al includes, apart from generally applied variables, the aspect of a possible increased risk of hereditary CKD and the degree of hematuria (273). This prognostic model was shown to be applicable to predict the 10-year risk of ESRD in a nation-wide unselected cohort of Norwegian IgAN patients and predicted also which patients had the highest risk of developing ESRD after 10 – 20 years of follow-up as well as all-cause mortality risk (274). Disadvantages with this scoring system are: the complexity of variables included, posing a problem with missing data, and that it was not based on the new Oxford-classification of histopathological findings.

The simpler Absolut Renal Risk (ARR) score, presented by Berthoux et al (120) allows for the increased mortality risk in CKD patients by predicting the 10- and 20-year cumulative incidence of dialysis or death, similar to the Framingham risk score for cardiovascular events and death. The score is derived by counting the risk factors (1) arterial hypertension, (2) proteinuria $\geq$ 1 g/ day and (3) severe pathologic lesions [global optical score, GOS $\geq$ 8, according to their French scoring system as presented in 1990 (83, 275)]. The authors analyzed a prospectively established

database of 332 locoregional IgAN patients, optimally treated to achieve a blood pressure (BP) target of ≤ 130/80 mmHg and to reduce proteinuria below 1 g/day. Importantly, they could demonstrate an improvement in the survival of patients with BP at target. They also showed that the survival curves for patients that achieved reduction of proteinuria were similar to those with no or low proteinuria at baseline. Control of hypertension and proteinuria had additive effects on survival. The substitution of GOS ≥ 8 by Oxford-MEST score of 3 to 5 was proposed in order to adapt this ARR score to the new Oxford classification.

To evaluate the predictive value of the adapted ARR score in our patient population, we analyzed clinical data from 72 IgAN patients, in whom the renal biopsy had been scored according to the Oxford-MEST classification for inclusion of the patients in the ongoing European multicenter VALIGA study (Validation of the Oxford Classification of IgA nephropathy). The patients had been followed for a median of 80 months (30 – 226 months).
Patients with an ARR score of 3 versus those with an ARR score of 0 – 2 had significantly increased risk of ESRD [HR 15.64 (95% CI 2.98 – 82.04), p = 0.001]. The number of the patients in the different ARR risk groups is shown in figure 11. Seven patients reached ESRD (CKD stage 5) during follow-up with five patients belonging to the highest risk group. No patient had died.

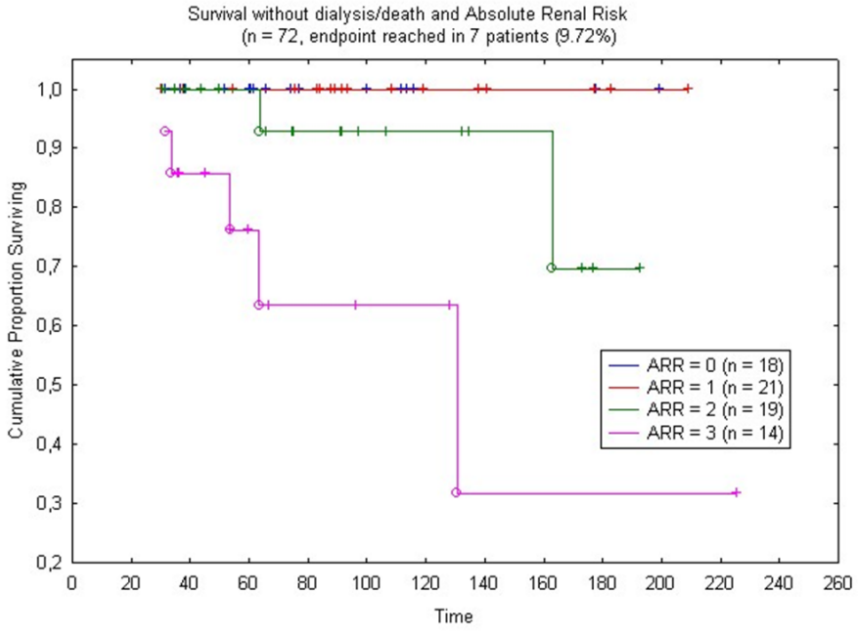


Figure 11. Kaplan-Meier curve showing that ARR risk scores are associated with renal outcome
For definition of "severe pathologic lesion" as one of the risk factors included in the ARR score, "global optical score ≥ 8" was substituted by "MEST ≥ 3" as proposed by Berthoux et al (120), using the new International Oxford classification instead of the local French scoring system. Other risk factors used for calculation of the ARR score are the presence of hypertension and of proteinuria ≥ 1 g/ day (here substituted by albuminuria ≥ 1 g/day) at diagnosis.

A different approach to the prediction of renal outcome is the formula developed by the Toronto group, presented by Bartosic et al (276). Instead of predicting outcome at diagnosis, they computed blood pressure (BP) and daily proteinuria during follow-up and found that, from these parameters, prediction of the yearly eGFR slope later in the disease course could be safely derived after three years of follow-up.

Toronto formula: ((TA proteinuria*(-0.13) + TA MAP*(-0.016) + 1.57))*12.

This formula has been validated in a European patient cohort in which the actual rate of progression could be predicted within 4 ml/min/year in 75% of subjects, predicting patients with the most rapid deterioration with the greatest accuracy (277).

To explore the applicability of the ARR score and the Toronto formula in some individual patients from our department we investigated clinical baseline and outcome variables in patients with IgAN and no concomitant disease that had been included in the VALIGA study. In this group, we compared the patients with the renal biopsy classified as M1E0S1T0 or M1E0S1T1 respectively, according to the Oxford classification, thus differing only with regard to the presence or absence of ≥ 25% and < 50% of tubular atrophy/ interstitial fibrosis. This discriminating variable was chosen due to the high impact generally found for tubulointerstitial changes in risk predicting models.
Figure 12 illustrates the clinical course in these patients with regard to the renal parameters s-creatinine and albuminuria and with regard to blood pressure during follow-up.

One patient in the M1E0S1T0 group (*male age 18*) experienced rapid renal function decline after an initially slow progression rate and a period of proteinuria remission that had occurred despite inadequate blood pressure control. There was also significant weight gain during the follow-up period from BMI 20 to 26. The other five patients had slow progressive or stable disease. None of these patients had received immunosuppressives.

Two patients in the M1E0S1T1 group developed ESRD and four of seven patients in this group had an annual eGFR loss of at least 2 ml/min/1.73 m². Only '*female age 42'* had been treated with immunosuppressives. She was put on an ACE inhibitor directly after the renal biopsy in 1995 and a six-month course of oral Prednisolone was introduced after two years due to a doubling of s- creatinine. A repeated biopsy was performed in 1999, still showing active crescentic lesions, as well as advanced chronic lesions, and a new course of Prednisolone treatment was introduced which could however not prevent the progression to ESRD. Albuminuria and MAP during follow-up never reached current treatment goals.

Tables 8 and 9 describe baseline and follow-up clinical characteristics of all the cases presented in the graphs. ARR score and predicted annual eGFR slope by the Toronto formula were calculated. We also applied our risk score, evaluated during follow-up as proposed in **paper IV,** including the parameters: hypertension, albuminuria ≥ 1g/day and FGF23 levels > 23 RU/mL (here called FGF23 risk score).

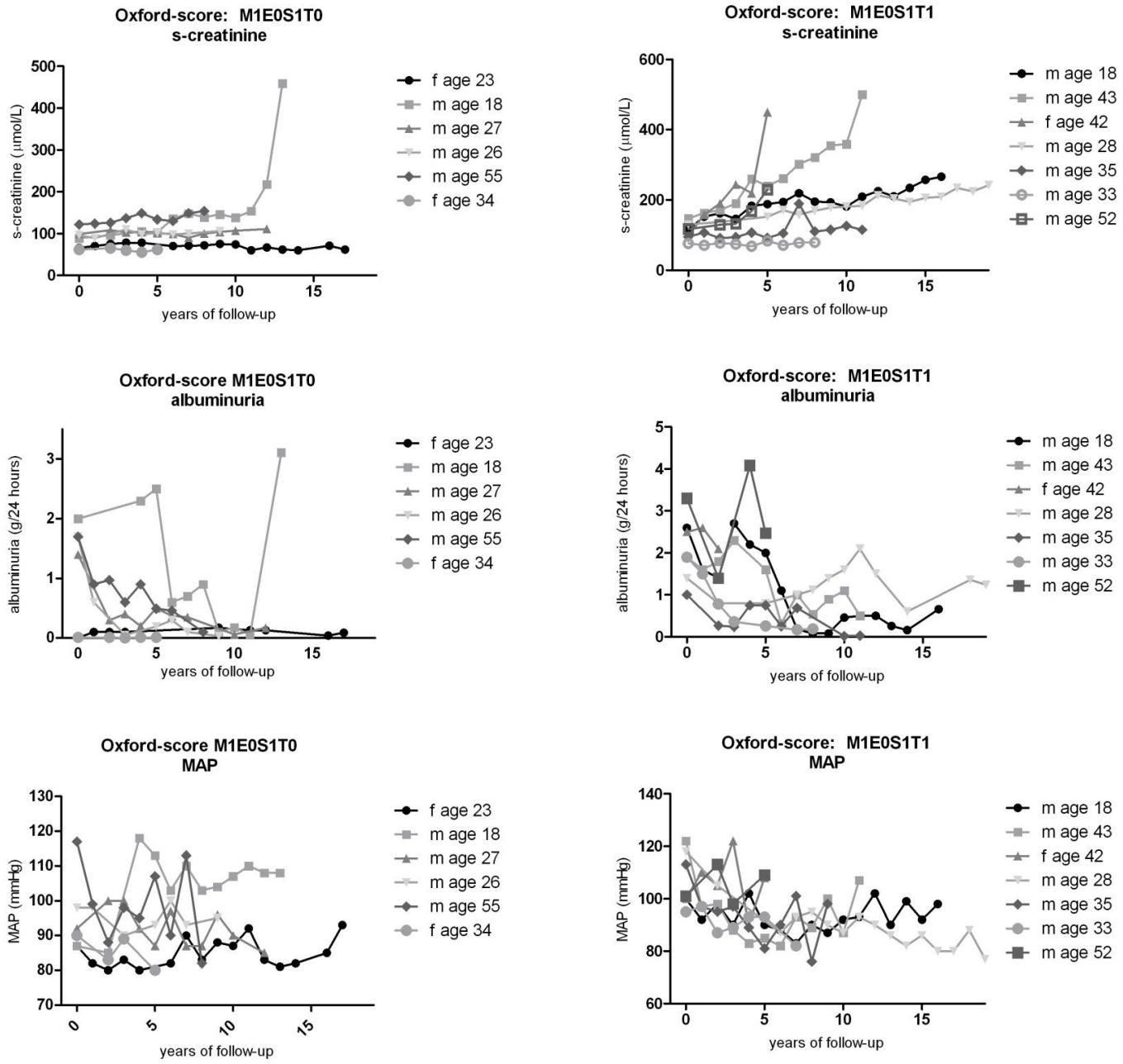


Figure 12 .Patients with IgAN and an Oxford- biopsy score of M1E0S1T0 or M1E0S1T1 The cases are labeled with sex (m, male; f, female) and the age at the time of renal biopsy

Table 8: Patients with the renal biopsy scored as Oxford-M1E0S1T0

	f age 23	m age 18	m age 27	m age 26	m age 55	f age 34
ACEI/ARB (% of time)	0%	**100%**	73%	100%	**99%**	0%
No of aHTs at last FU	0	**4**	1	2	**1**	0
Fam history of renal disease/HT	no / no	**no / yes**	no / yes	yes / yes	**no / no**	no / no
Year of onset/ biopsy	’92 / ’94	**’92 / ’96**	’98 / ’99	’89 / ‘00	**’93 / ‘03**	’05 / ‘06
% crescents in biopsy	0%	**9%**	27%	0%	**0%**	0%
BMI (biopsy – last FU)	22 - 24	**20 - 26**	24 - 24	26 - 26	**26 - 26**	19 - 19
Remission of hematuria	yes	**no**	no	yes	**yes**	no
Smoker	no	**no**	no	no	**no**	no
CKD stage at diagnosis	1	**1**	2	2	**3a**	1
ARR score[1]	0	**2**	1	1	**2**	0
FGF23 risk score[2]	0	**3**	0	1	**3**	0
Time from biopsy to FGF23 measurement (years)	7	**4**	1	3	**0**	2
Predicted[3]/actual eGFR slope	3.0 / 0.5	**-7.4 / -4.6**	-0.9 / -0.7	0.3 / -1.4	**-0.7 / -1.5**	2.3 / 0.9
Dialysis or Death / CKD stage 3b+	no / no	**yes / yes**	no / no	no / no	**no / yes**	no / no

Table 9: Patients with the renal biopsy scored as Oxford-M1E0S1T1

	m age 18	m age 43	f age 42	m age 28	m age 35	m age 33	m age 52
ACEI/ARB (% of time)	**93%**	**98%**	**99%**	**100%**	98%	100%	**100%**
No of aHTs last FU	**1**	**1**	**4**	**2**	2	1	**5**
Fam history of renal disease/HT	**no / no**	**yes / yes**	**-**	**no / -**	no / no	no / no	**no / no**
Year of onset/ biopsy	**’94 / ‘95**	**’94 / ‘95**	**’95 / ‘95**	**’70 / ‘92**	’99 / ‘00	’85 / ´03	**’98 / ‘06**
% of crescents in biopsy	**0%**	**0%**	**22%**	**0%**	0%	0%	**0%**
BMI (biopsy – last FU)	**23 - 27**	**24 - 24**	**22 - 22**	**30 - 29**	30 - 33	19 - 19	**31 - 32**
Remission of hematuria	**no**	**-**	**-**	**no**	yes	yes	**no**
Smoker	**no**	**no**	**no**	**no**	no	no	**no**
CKD stage at baseline	**2**	**3a**	**3a**	**3a**	2	1	**3a**
ARR score[1]	**2**	**3**	**3**	**3**	3	2	**3**
FGF23 risk score[2]	**2**	**3**	**3**	**1**	2	1	**3**
Time from biopsy to FGF23 measurement (years)	**2**	**1**	**2**	**4**	2	1	**2**
Predicted[3]/ actual eGFR slope	**-2.0 / -2.1**	**-2.3 / -3.1**	**-6.5/-10.3**	**-2.6 / -1.6**	0.0 /-2.1	-0,2 / -1.3	**-5.7 /-6.0**
Dialysis or Death / CKD stage 3b	**no / yes**	**yes / yes**	**yes / yes**	**no / yes**	no / no	no / no	**no / yes**

[1]ARR, adapted: MEST ≥ 3, presence of hypertension, albuminuria ≥1 g/24h; [2]presence of hypertension, , albuminuria ≥ 1 g/24h, FGF23 > 23 RU/ml; [3]predicted by the Toronto formula (276); FU, follow-up; HT, hypertension; aHT, antihypertensives. Cases with ESRD or CKD stage 3b+ are presented in bold.

These examples illustrate the importance of continuous re-evaluation of an individual patients' risk profile during follow-up and the usefulness of currently available risk prediction models. Though not including histopathological findings, the Toronto formula as well as our proposed FGF23 risk score may to some extent mirror the pre-existing renal damage.
The value of repeated eGFR measurements during the first years of follow-up in risk prediction has also been shown earlier by Rekola et al in a Swedish patient cohort (278). In this study, 50% of the patients with initial normal renal function and a negative eGFR slope of more than 1.1 ml/min/1.73m²/year had subnormal renal function during follow-up.

We could not use a combination of the biomarkers examined in the studies of this thesis for risk prediction analyses as these markers in the majority of patients had been analyzed in blood samples obtained at different time points during the follow-up period. Future studies will show which biomarkers explored world-wide are those that have the greatest contributory value in risk predicting models and clinical decision trees.

4 GENERAL DISCUSSION

4.1 METHODOLOGICAL CONSIDERATIONS

Study population and follow-up period
IgAN is a relatively rare disease, which impedes the possibility to increase patient numbers and improve statistical power in studies like ours. Research on IgAN is further embarrassed by individual variability in disease manifestations, leading to a diagnosis in different CKD stages and little knowledge of the duration and progression rate from disease onset. Furthermore, in the majority of cases, IgAN progresses slowly, thereby necessitating a long follow-up period to correctly identify patients with severe renal outcome.
The clinical characteristics of IgAN patients at Karolinska University Hospital are comparable to patient cohorts described from other European centers (120, 128, 155, 274, 277, 279). Some of the differences seen can be ascribed to the catchment area of the different hospitals, local policies for patient referral and the performance of a renal biopsy, treatment traditions and follow-up routines.
Benefits of our study cohort are the high attendance of the patients participating in the study, the non-selective referral policy to our department by means of disease severity, and our possibility to follow these patients during many years, irrespective of their disease state, which reduces the number of patients lost to follow-up. All these factors make the patient population of **study III and IV** highly representative of the whole spectrum of the disease and its prognosis and we met the qualifying criteria described by d'Amico for this kind of studies (280).

Definition of renal outcome
The optimal definition of renal outcome for the identification of patients with IgAN at high risk for severe disease progression has yet to be identified. During the past decades, different surrogate outcome variables have been used to overcome the limitations in follow-up periods for clinical and observational studies. In our analyses, we used varying outcome definitions as described on page 22, to some extent following trends in the literature when these studies were planned. CKD stage 3b+ was introduced as a new endpoint-definition in the analyses described in the 'Additional results' section as it is hypothesized that renal disease progression in patients with more advanced renal failure may be less specific to the underlying disease. This outcome definition might be worth evaluating as a surrogate marker for interventional studies with regard to disease-specific treatment.
The rate of annual renal function decline (eGFR slope) was used as a measure of the severity of renal disease progression in **studies III and IV** in addition to hard or surrogate endpoints. It is generally accepted that progression to renal failure in IgAN is linear (281) with a higher eGFR slope in patients with preceding glomerular and interstitial injury, consistent with the hyperfiltration hypothesis of renal disease progression earlier described in this thesis. The annual eGFR decline is widely used as an outcome variable in recent studies (89, 276, 277, 281). However, some patients have accelerated renal function decline that can appear early or late in the disease course, as illustrated by the individual cases described in the 'Additional results' section. This could be caused by crescent formation, by increasing vulnerability of remnant nephrons due to oxidative stress and inflammatory mechanisms associated with advanced renal failure, or by currently unknown mechanisms. Notably, the renal progression rate is sometimes

slow, despite the presence of advanced renal failure. This is presumably more often the case in patients diagnosed at higher age.

Statistical methods
Survival analysis was applied in **studies II, III and IV** as in most of the studies in the literature due to 'time' being an important factor for renal outcome in IgAN. The relatively low number of severe outcomes limited the validity of heavily adjusted models for the exploration of possible confounders, and contributed to wide confidence intervals in some of our calculations.
To deal with the non-normal distribution of the investigated potential biomarkers, threshold models were used in studies II, III and IV. In study IV and in our additional analyses we also used log-transformed values as continuous variables in the linear regression models.
In the multivariable regression models in study IV, clinically relevant increases in the respective variables were used for stratification.

Estimation of glomerular filtration rate
In the field of nephrology in adults, the gold standard for GFR measurements in Sweden nowadays is the clearance of iohexol or, in the case of intolerance, the clearance of Cr-EDTA. However, these methods are expensive and time-consuming and thus inconvenient in clinical practice and for yearly evaluation of renal function. The MDRD equation for estimation of GFR, based on s- creatinine values, was developed 1999 from the Modification of Diet in Renal Disease study, which consisted of 1628 non-transplanted CKD-patients with non-diabetic kidney disease. The formula was re-expressed in 2006 for standardized creatinine calibration methods (226, 282) and is widely used in research. Estimated GFR by the MDRD formula and GFR returned from the clearance of iohexol highly conform in the range of CKD stages 3 – 5 whereas the MDRD-eGFR formula to some extent underestimates kidney function in CKD stages 1 – 2. The recently launched CKD-EPI equation is considered to provide a more exact estimate of kidney function in the range of CKD stages 1 – 2. All creatinine-based estimations of GFR can be incorrect in the case of extremities in age or body size, in pregnancy, in severe malnutrition or obesity, in vegetarian diet, in skeletal muscle diseases and paraplegia and in rapid changes in renal function.
In **study IV** we performed a secondary analysis of our data estimating GFR by the CKD-EPI formula with regard to the fact that 68% of the patients had CKD stage 1 – 2 at baseline. In fact, using the CKD-EPI equation we identified additional patients with more rapid renal function decline. Thus, misclassification of patients with severe disease was further reduced and the power of the statistical analysis slightly improved.

4.2 FINDINGS AND IMPLICATIONS

Main risk factors for renal function loss in IgAN
In most reports, including those presented in this thesis, the risk factors with the highest impact on renal outcome in IgAN were those associated with advanced renal damage such as reduced glomerular filtration rate, established hypertension, persistent high-grade proteinuria and, histologically, the severity of glomerular sclerosis and tubular atrophy/ interstitial fibrosis (111, 283). Renal proliferative changes only slightly improved the predictive value of these factors, which in part may be biased by effects of treatment, given the limitations of current therapy in modifying chronic sclerotic and fibrotic lesions compared with the potential of antihypertensive

and/ or immunosuppressive therapy to modify mesangial and endocapillary hypercellularity (89, 96, 284-286). Failed or insufficient response to treatment with blockers of the angiotensin-aldosterone system and immunosuppressives with regard to blood pressure and albuminuria most probably reflects preexisting renal damage, providing some explanation to the high predictive value of follow-up measurements of these variables for disease outcome.

Additional risk factors for CKD progression that can be targeted by current treatment
Our studies add to the knowledge about the impact of both traditional and non-traditional cardiovascular risk factors on renal disease progression in IgAN. Some of these factors can be targeted by optimal supportive treatment to prevent or at least attenuate the development of advanced renal damage.
We identified **activated monocytes** as one possible source of **oxidative stress** in IgAN and an *in vivo* inhibitory effect of atorvastatin on monocyte activation (**study I**). Oxidative stress is one of the established non-traditional risk factors associated with cardiovascular disease and seems to occur early in CKD (229, 287). The critical role of oxidative stress in the pathogenesis of CKD-related atherosclerosis involves an enhanced effect of systemic inflammation on vascular calcification (288-290) and inflammation may also deplete antioxidants (291). In the kidney, reactive oxygen species (ROS) can damage biological macromolecules and contribute to cell apoptosis.
We further demonstrated an association of the apolipoprotein B/apolipoprotein A-I ratio with renal outcome, which was found to be independent of renal function and, importantly, also independent of serum albumin that biologically can influence the **lipid-balance** (**study II**). The negative impact of apolipoprotein B on cardiovascular disease is well established, as reviewed by Olofsson and Borén (292) and included in international guidelines. The apoB/apoA-I ratio is supposed to give additional information on the balance of atherogenic and anti-atherogenic lipids, though the use of ratios instead of isolated values as biomarkers for risk assessment is not generally accepted.
In IgAN, fluvastatin-treatment improved proteinuria in parallel with improvements in the lipid profile (293, 294). In diabetic patients, lipid-lowering therapy is the most effective step to prevent cardiovascular disease in multiple disciplinary treatment approaches (295). In the recently published large SHARP trial, treatment with a combination of simvastatin and ezetimibe reduced the risk of major atherosclerotic events but not the risk of ESRD or a doubling of baseline creatinine (296). Smaller studies suggest that the renoprotective effect of statin-treatment may be more pronounced in individuals with lower eGFR and an **'inflamed state'**, characterized by higher serum levels of CRP and soluble tumor necrosis factor receptor 2 (TNFR II) (269, 297).

Apoliprotein A-I has been proposed as **a possible link between infection and chronic inflammation**. Burger and Dayer showed that contact-mediated activation of monocytes by stimulated T lymphocytes triggers the production of large amounts of TNFα and interleukin-1β (IL-1β), both of which are involved in chronic inflammation. Apolipoprotein A-I was found to be a specific inhibitor of this reaction, which might explain some of its anti-inflammatory effects (298).

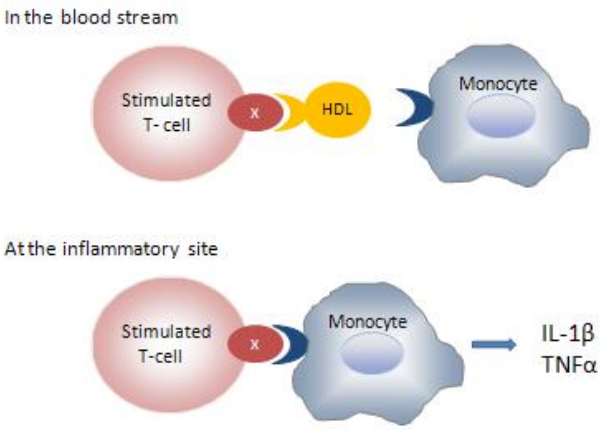


***Figure 13. Mode of action of apo A-I. In normal condition, the level of HDL in the blood stream is sufficient to inhibit the triggering of cytokines in monocytes mediated by direct cellular contact with stimulated T lymphocytes. HDL-associated apo A-I binds the stimulating factor (X) at the surface of T lymphocytes hampering the binding of the latter with its specific receptor at the monocyte surface, thus inhibiting the production of TNF-α and IL-1β. Adapted with permission from* Burger and Dayer 'High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and inflammation?', Autoimmunity Reviews 1 (2002) 111-117.**

T-lymphocytes are involved in the pathogenesis of IgAN and a continuous **T-cell stimulation** may contribute to persistent inflammatory activity and faster disease progression in IgAN. Our findings of the association of sIL-2Ra levels with renal disease progression and with a higher degree of tubular atrophy and interstitial fibrosis support this view. This is in line with the current concept that the progression of renal fibrosis is associated with the presence of infiltrating mononuclear cells, predominantly T lymphocytes and macrophages (204, 242-244, 299, 300) and that T-cells are involved in atherosclerotic processes (301-303). Notably, not only treatment with immunosuppressives but also treatments with ACEIs and statins have been shown to influence T lymphocyte activity (304-306). The observational character of our study and the limited number of patients on lipid-lowering therapy or with a history of immunosuppressive treatment did not allow any statistical analysis of the effect of treatment on either sIL-2Ra levels or outcome. This will be an interesting question for future randomized trials.

Soluble TNFRs I and II as well as sIL-2Ra are small molecules the size of 55 kDa, 57 kDa and 45 kDa respectively and their rise in the serum of patients with CKD could be explained both by retention and increased production. As presented in the 'Additional Results' section, log TNFR I and log TNFR II correlated to logIL-2Ra and all three markers were associated with renal survival independent of the main progression risk factors namely baseline eGFR, time-averaged albuminuria and time-averaged MAP (Table 6). The independent predictive values of TNFR I and II and sIL-2Ra for renal disease progression in IgAN are new findings. They are in line with the hypothesis that plasma TNFR levels in patients with IgAN, and possibly also in patients with CKD due to other causes, are not only elevated due to retention when the glomerular filtration ratio falls but may also reflect an 'inflamed' state that increases in lower CKD stages and further promotes renal disease progression. Statins have been shown to reduce levels of tumor necrosis factor as well as MCP-1, TGFβ, IL-6 and NFκB (170, 307, 308), all involved in the inflammatory signaling pathways in progressive renal damage. Further studies are warranted to

establish whether the levels of IL-2Ra and TNFR I or II are appropriate considerations in the monitoring of the effect of treatment, aimed to reduce the inflammatory burden and to prevent the progression of renal failure.
Interestingly, a recent German pilot study showed that treatment with the herb wormwood (*Artemisia absinthium*) reduced proteinuria in IgAN patients who had proteinuria levels between 0.5 and 3.5 g/ day despite double RAAS blockade and who had not responded to fish oil treatment (309). The authors speculate that this effect might be the result of inhibitory effects of wormwood on TNFα and NFκB as has been shown in vitro and in patients with Crohn's disease (310-312).

The accelerated atherosclerosis process in CKD is not only caused by traditional and non-traditional cardiovascular risk factors as in the general population, but also by **vascular calcification** related to disturbances in renal function and the CKD-MBD complex. Hyperphosphatemia, dietary phosphate load, vitamin D insufficiency and secondary hyperparathyreoidism, probably altogether reflected by FGF23 values, increase the risk of vascular calcification and CVD. Our study shows a predictive value of FGF23 for persistent proteinuria and CKD progression in IgAN and contributes to the concept that disturbances in mineral metabolism start early in the course of CKD and should be targeted by dietary recommendations and medication. Herein, FGF23 may serve as a marker for decision-making on therapeutic approaches as its levels appear to be elevated prior to any detectable abnormalities in calcium, phosphorus, PTH or vitamin D metabolism (313).
In IgAN, **treatment with vitamin D** has been shown to reduce proteinuria alone or in combination with blockers of the RAAS (314, 315). Experimental studies provide support for 1.25 dihydroxy vitamin D-mediated downregulation of renin and the RAAS via activation of the vitamin D receptor (316). It has been suggested that vitamin D may be consumed during inflammatory processes, which is supported by the findings that statin-treatment led to increased levels of 25 (OH) and 1.25 (OH) vitamin D, possibly through anti-inflammatory actions (317).

Metabolic factors beyond calcium, phosphate and vitamin D have been proposed as determinants of FGF23. In our study, FGF23 correlated with BMI and hypertension. In addition to these variables, Gutierrez et al also reported on associations with other traditional cardiovascular risk factors such as smoking and dyslipidemia (318).
Uric acid levels have been associated with urinary phosphate excretion as well as with FGF23 levels in serum, suggesting that circulating FGF23 levels and uric acid may be an early indicator of high protein and phosphate intake (318, 319). Elevated uric acid levels have been shown to be an independent risk factor for CVD, diabetes, insulin resistance, metabolic syndrome, gout and uric acid stone formation and are associated with excessive purine consumption (320). Hyperuricemia has usually been interpreted as a marker of renal dysfunction but there is now emerging evidence of the pathogenetic role of uric acid in hypertension and renal disease progression with activation of the renin-angiotensin-system as one of the mechanisms (321, 322). This view will have to be supported by larger interventional studies. In IgAN, hyperuricemia was an independent risk factor for renal disease progression and correlated with the severity of tubulointerstitial changes (128, 323). *In vitro* studies have demonstrated that uric acid can directly promote human T-cell activation and enhance antigen-specific immune responses (324).

The impact of **obesity** on the progression of CKD has been reviewed by Chalmers et al (325). Obesity is characterized by similar cardiovascular risk factors as those found in CKD, including oxidative stress, lipid abnormalities and persistent inflammation. These mechanisms may act synergistically on renal and cardiovascular outcome in CKD. Tanaka et al have reported on significant thickening of the glomerular basement membrane in obese patients with IgAN (326, 327), which presumably contributes to increased proteinuria. This is supported by earlier studies showing that weight loss has beneficial effects on proteinuria (328, 329). Despite BMI not being a predictive factor for renal outcome in IgAN in our studies, clinical observations suggest this to be true in some individual patients. Changes in body weight during follow-up, as indicated in the clinical cases in tables 8 and 9 might have an impact on renal outcome both in the case of weight gain and when BMI decreases as a consequence of lifestyle changes or gastric surgery.

Renal histopathological markers of inflammation might improve the predictive value of histopathological findings for renal disease progression and response to therapy. In the current version of the International Oxford classification immunostaining methods were not included, as the goal was to develop a classification that is simple and can be widely applied in clinical practice. For future research purposes, however, more information on disease promoting mechanisms is proposed to be obtained by such methods. A reevaluation of renal biopsies from patients included in the earlier performed randomized controlled intervention trials may provide valuable information on histopathological and immunological markers for changes that are susceptible for treatment.
Recommendations for the performance of **repeated biopsies** are lacking and the risks associated with this procedure need to be considered.

Despite the strong association found between the degree of tubulointerstitial fibrosis and worse prognosis in IgAN and other forms of renal disease, there is nevertheless some hope provided from experimental studies that **fibrotic and even sclerotic histopathological changes might not be as irreversible as previously thought (330, 331)**. RAAS blockade decreased fibrosis both in animal models (330) and in patients with mesangial proliferative glomerulonephritis (332). As reviewed by Chatziantoniou and Dussaule, future therapies like growth factor inhibitors may be a complement to conventional drugs with anti-fibrinogenic potential such as RAAS blockers and statins (331).

What is the **impact of persistent severe microhematuria on renal disease progression in IgAN?** In the risk prediction score and the decision tree model proposed by Goto et al, the degree of microhematuria has been included as one of the variables (273, 333). However, until now, the prognostic impact of persistent or increasing degrees of microscopic hematuria on renal outcome in IgAN has been suggested but is not yet proven (106, 271, 334). The prognostic value of urinary erythrocytes may be greater in the early stages of the disease (279). There are pitfalls in quantifying hematuria which may contribute to the inconsistent results. Moreover, hematuria may be both an indicator of the severity and chronicity of the underlying glomerular injury and, at the same time, a promoter of tubular injury through hemoglobin-induced oxidative stress (335). In the field of vasculitis and lupus nephritis, urinary dipstick findings and/or urinary cell counts, in addition to other clinical markers and histopathological findings, are routinely integrated in the assessment of inflammatory activity and recommendations for treatment. This is

still not the case for IgAN. Disappearance of hematuria, on the other hand, has been added to the definition of complete remission also in this kind of glomerular disease (336).
In our studies we did not include the degree of hematuria in the prediction models due to the lack of clinical data. During the first years of this follow-up study, urinary dipsticks or cell counts were not routinely included in the parameters assessed alongside follow-up visits. There are also difficulties in the interpretation of these findings in the presence of hypertensive vessel changes, frequently found in the renal biopsy specimens of patients with IgAN.

In **summary**, systemic inflammation as well as metabolic factors are involved in renal disease progression in IgAN and can be targeted by current therapy. There is a great need for further studies evaluating serum, urinary and histological inflammatory markers regarding their usefulness in the monitoring of treatment in clinical practice. Treatment with statins as well as vitamin D supplementation have led to the amelioration of proteinuria, independent of ACEI/ARB treatment, probably through analogous effects on inflammatory pathways (337). Evidence is still lacking to allow for the inclusion of these medications in general treatment recommendations targeted at lowering proteinuria. Nevertheless, the increased risk of CVD in patients with reduced renal function usually implies indications for this kind of treatment beyond the indication of proteinuria. The additional therapeutic value of uric acid lowering treatment, antioxidants, herbs and dietary recommendations in the attenuation of renal disease progression has to be further proven.

Hereditary risk factors – the role of hypertension
Hypertension is highly prevalent not only in patients with IgAN but also in their closest relatives as shown in our patient cohort. In a small French study, a family history of hypertension was linked to nephrosclerotic changes in renal biopsies from patients with IgAN and was an indicator for an unfavorable prognosis (338). This opens for the speculation that a **hereditary predisposition for hypertension** may increase the vulnerability for IgAN. Alternatively, hypertension and IgAN are caused by similar predisposing factors. Interestingly, Yamamoto et al found an association of IgAN disease progression with gene polymorphisms linked to cardiovascular disease (339, 340).

Risk prediction in individual patients
All the risk factors and scoring systems described in this thesis only predict renal disease progression in IgAN to some extent. Additional disease promoting mechanisms still have to been explored as exemplified by the clinical cases where the renal disease is highly progressive despite optimized supportive therapy. Moreover, it is somewhat problematic to evaluate the value of a factor at baseline for prediction of later outcome if this factor is supposed to change during the follow-up period as is suggested for the variables apoB/apoA-I ratios, aIL2-Ra, FGF23 and markers of oxidative stress. This is illustrated by the now well-documented findings that the degree of albuminuria and blood pressure during follow-up has a considerably higher impact on renal outcome than baseline values and by the fact that the independent predictive value of proliferative histopathological findings in the Oxford classification was attenuated by adjustment for immunosuppressive treatment and could not be reproduced in several similar-sized validation studies (89, 91).

Due to the complexity of interacting mechanisms involved in the progression of CKD and CVD, a single biomarker cannot be expected to have more than modest value in the overall risk prediction of either renal or cardiovascular events. **Epidemiologic approaches that include a combination of multiple risk factors**, including metabolic parameters, are supposed to improve risk assessments and need to be further evaluated by larger clinical studies regarding their usefulness in the targeting and monitoring of treatment.
Low attendance to therapy and life-style recommendations always presents a complicating factor in a group of patients, which might be a special problem in young individuals affected by kidney disease, usually not associated with overt clinical symptoms early in the disease course. This should be overcome in most cases through the advantages of a long-lasting patient-doctor relationship in this kind of chronic, slowly progressive disease and through a team-approach including nurses, dieticians, physiotherapists and psychologists in the clinical care.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

A diagnosis of IgAN implicates a considerable risk of chronic renal failure and premature cardiovascular disease. Until further understanding of disease mechanisms and how those can be addressed or even prevented by disease-specific therapy, a multidisciplinary treatment approach is needed to attenuate renal disease progression and to reduce cardiovascular morbidity and mortality. The findings in the present work contribute to the conception that similar mechanisms are involved in the development of atherosclerosis and glomerulosclerosis and that persistent activation of the immune system has an impact on the rate of renal function decline. Moreover, in a pilot study, we illuminate one of the possible mechanisms by which statin treatment could attenuate oxidative stress in chronic inflammatory renal disease.

Specifically, based on the different study results, we conclude that:

- Activated circulating monocytes might be one of the sources of oxidative stress in chronic inflammatory kidney disease.

- One of the pleiotropic effects of statins, regarding the protection from progressive atherosclerosis and glomerulosclerosis, seems to be a direct effect on monocytes by reducing their production of reactive oxygen species.

- Measurement of the apolipoprotein B/apolipoprotein A-I ratio, easily applied in clinical practice without the need of a fasting condition, can help to identify patients with increased risk of renal deterioration. This should lead to a multidisciplinary treatment approach targeted at this variable: dietary recommendations, lipid lowering medication, excess weight reduction, physical exercise, smoking cessation among others.

- A high plasma level of sIL-2Ra is predictive of renal disease progression in IgAN, providing further support for the view that IgAN is a T cell-driven disease. Further studies are warranted to evaluate whether sIL-2Ra levels could be a useful marker in the monitoring of effect-of-treatment, aimed to prevent the progression of interstitial fibrosis and progressive glomerulosclerosis in this patient group.

- Circulating FGF23 is associated with albuminuria and CKD progression in patients with IgAN. This study extends the role of FGF23 as a predictive biomarker for CKD progression exclusively in the IgAN population and poses the question whether FGF23 modifies albuminuria as part of an ‘off-target’ effect that has been postulated, but not yet proven.

- Evaluation of risk factors in our studies confirms earlier findings establishing time-averaged proteinuria as being the strongest variable that influences the rate of renal disease progression.

Finally: Despite improvements in the care of patients with IgAN during the last decades, due to increasing knowledge about mechanisms involved in renal disease progression in general and, possibly, due to a wider use of immunosuppressives, these individuals still constitute a high percentage of all patients in renal replacement therapy. Much more research is needed to find ways of specifically intervening disease initiating and maintaining mechanisms. Newer treatment approaches, currently under investigation, target complement, Toll-like receptors, antigen-presentation by dendritic cells or proteasomes, NfκB, antigen-producing B-cells, cytokine signaling and cell proliferation (341). The genetic profile not only may give a hint about adequate treatment approaches but also may reveal different mechanisms predominantly involved in the initiation and maintenance of IgAN in different patients and different ethnicities.

Further analyses of the yearly collected blood and urine samples in our study population will increase our knowledge on the value of repeated measurements of clinical biomarkers in the assessment of the renal and cardiovascular risk in IgAN patients and the monitoring of optimal treatment. This will also be reinforced by our planned investigations of the vascular status of patients with IgAN at different time points during the disease course, in correlation to clinical and biochemical findings.
These kinds of investigations are supposed to help in the detailed characterization of patients to be included in interventional studies for the evaluation of more specifically targeted and tailored therapy. In the future, hopefully, a diagnostic kit of biomarkers that are available in clinical practice will help the clinician to decide on more individualized therapy aimed to prevent both renal failure and cardiovascular disease in patients with IgAN.

As IgAN is a relatively rare disease with a variety of clinical manifestations, new landmarks can only be reached through national and international collaboration. The increasing size of the International IgA Nephropathy Network, founded in 1987, and the ERA-EDTA initiated Immunonephrology Working Group under the leadership of Professor Rosanna Coppo have already led to a number of European and International collaboration projects in IgAN. Together, these initiatives have the potential to improve our diagnostic and therapeutic opportunities in the field of IgAN, similar to the work done in other forms of inflammatory renal disease such as ANCA associated vasculitis or systemic lupus erythematosus.

6 SUMMARY IN SWEDISH/ POPULÄRVETENSKAPLIG SAMMANFATTNING

Glomerulonefrit (GN) är huvudorsaken till kronisk njursvikt hos patienter som är dialysberoende eller njurtransplanterade och IgA nefrit (IgAN) är den vanligaste enskilda GN diagnosen. Patienter med nedsatt njurfunktion och/eller proteinuri har en ökad risk för tidig utveckling av hjärt-kärlsjukdom. Eftersom liknande patogenetiska mekanismer anses vara involverade i fortskridandet av ateroskleros och glomeruloskleros, var syftet med denna avhandling att studera, till vilken grad kända traditionella och icke-traditionella kardiovaskulära riskfaktorer också indikerar en ökad risk att utveckla njursvikt hos patienter med en enhetlig diagnos av IgAN.
Kronisk inflammation betraktas som en viktig bidragande orsak till fortskridande njurskada. Vid IgAN har störningar i det medfödda och det förvärvade immunsystemet påvisats där T-lymfocyter har en betydande roll. Vi har därför också undersökt om plasma-nivåer av löslig interleukin-2 receptor (sIL-2Ra), en föreslagen markör för kontinuerlig T-cells stimulering, kan prediktera njurfunktionsförlust vid IgAN.

Studie I är en pilot studie som undersöker aktiverade monocyter som en potentiell källa till oxidativ stress vid IgAN. Monocyter i perifert blod från 16 patienter med IgAN och 16 friska kontrollpersoner stimulerades *in vitro* och produktionen av syre-radikaler mättes med hjälp av flödescytometri. Patienterna behandlades därefter under en månad med en daglig dos av 20 mg atorvastatin, varefter monocyternas produktion av syre-radikaler efter *in vitro* stimulering kvantifierades på nytt. Vi fann att monocyter från patienter med IgAN uppvisade en högre produktion av syre-radikaler jämfört med celler från friska kontroller och att statin-behandling ledde till en reducering av densamma.

I **studie II** undersökte vi 70 patienter med IgAN och kronisk njursvikt i stadium 1 – 3 [beräknad glomerulär filtrations hastighet (eGFR) > 30ml/min/1,73m²] och 70 ålders- och könsmatchade friska personer med avseende på kvoten av apolipoprotein B och apolipoprotein A-I (apoB/apoA-I) i blodet, vilken avspeglar balansen mellan de blodfetter som bidrar till, och de som motverkar, aterosklerosutvecklingen. Patienter med IgAN hade högre serum-nivåer av apoB/apoA-I i jämförelse med de friska kontrollerna och en apoB/apoA-I kvot över de föreslagna gränsvärdena av 0,9 för män och 0,8 för kvinnor var förknippad med en ökad risk för utveckling av terminal njursvikt hos IgAN patienterna, efter en uppföljningsperiod av i genomsnitt 3,8 år.

Studie III omfattade 194 patienter med IgAN, av vilka 179 patienter med kronisk njursvikt i stadium 1 – 4 (eGFR > 15ml/min/1,73m²) hade följts kliniskt under upp till 16 år (medianvärde 52 månader). Plasmakoncentrationen av sIL-2Ra var högre hos patienterna jämfört med hos 84 friska kontrollpersoner. Nivåer av sIL-2Ra i högsta tertilen indikerade en ökad risk för njurfunktionsförsämring hos IgAN patienterna och sIL-2Ra nivåerna korrelerade även till den årliga förlusten av eGFR. Hos 51 patienter hade njurbiopsin genomförts maximalt 2 år före analysen av sIL-2Ra och granskats i enlighet med den nya internationella Oxford-klassifikationen. I denna subgrupp av patienter korrelerade sIL-2Ra nivåerna med förekomsten av > 25% tubulär atrofi/ interstitiell fibros (graderad som T 1 – 2 enligt Oxford- MEST klassifikationen), vilket var statistiskt signifikant även efter justering för njurfunktionen.

Studie IV inkluderade 180 IgAN patienter med kronisk njursvikt i stadium 1 – 4 vilka hade följts prospektivt (median 55 månader). Vi analyserade serum-nivåer av det fosfat- reglerande hormonet FGF23 (fibroblast growth factor 23), en nyckelspelare i symptomkomplexet av kronisk njursvikt och medföljande störningar i mineral-metabolism och skelettuppbyggnad. FGF23 koncentrationen var associerad med en allvarligare grad av njurfunktionsförsämring och korrelerade även till graden av albumin-utsöndringen i urinen vid inklusion och under uppföljningstiden.

Sammanfattningsvis kunde vi visa att flera riskfaktorer för ateroskleros också är riskfaktorer för njurfunktionsförsämring hos patienter med IgAN, oberoende av de kända huvudsakliga riskfaktorerna proteinuri och högt blodtryck. En kontinuerlig aktivering av T- celler är en möjlig bidragande faktor till fortskridande njursvikt vid IgAN. De undersökta potentiella biomarkörer kan vara av värde för att monitorera behandlingen hos patienter med IgAN.

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